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This thesis is based on the following papers. In the text they are referred to by their Roman numerals.

I Kidney function during hydropenia and water diuresis in patients with idiopathic recurring nephrolithiasis.
Backman, Ulla.
Scand J Urol Nephrol. Submitted for publication 1976.

II A short duration renal acidification test.
Backman, Ulla, Danielson, B.G. & Sohtell, M.
Scand J Urol Nephrol, Suppl. 35, 33, 1976.

III Urine acidification capacity in renal stone formers.
Backman, Ulla, Danielson, B.G. & Sohtell, M.
Scand J Urol Nephrol, Suppl. 35, 49, 1976.

IV Bicarbonate re-absorption in renal stone patients with acidification defects.
Backman, Ulla, Danielson, B.G. & Sohtell, M.
Scand J Urol Nephrol, Suppl. 35, 62, 1976.

V Urinary excretion of β_2-microglobulin in renal stone patients under normal conditions and during acidosis and alkalosis.
Backman, Ulla, Danielson, B.G. & Sohtell, M.
Scand J Urol Nephrol, Suppl. 35, 76, 1976.

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LIST OF SYMBOLS

GFR	glomerular filtration rate, ml/min
RPF	renal plasma flow, ml/min
$T^c_{H_2O}$	tubular reabsorption of water, ml/min
C_{H_2O}	free water clearance, ml/min
C_{Na}/C_{In}	fractional excretion of sodium, per cent
$C_{HCO_3^-}/C_{In}$	fractional excretion of bicarbonate, per cent
RTA	renal tubular acidosis
TA	titratable acid, mmol/kg b.w. and hour
P_{CO_2}	partial pressure of carbon dioxide
C_{PAH}	clearance of para-amino-hippuric acid, ml/min
BE	base excess, mmol/l
$T_m HCO_3^-$	tubular transfer maximum for bicarbonate, mmol/100 ml GFR
p	probability
p:n.s.	not significant
$0.05 > p > 0.01$	probably significant
$0.01 > p > 0.001$	significant

INTRODUCTION

Genetic, metabolic and epidemiological studies of renal stone disease indicate that intrinsic and extrinsic factors operate together over a period of time in the development of renal stones (Andersen, 1973; Resnick, Pridgen & Goodman, 1968; Coe, 1974; Pak, Ohata, Lawrence & Snyder, 1974). Calcium oxalate and calcium phosphate stones are the most common and present the greatest problems, as the pathogenesis is unclear and the treatment often unsatisfactory (Clark & Nordin, 1968). One expression of this is the high recurrence rate (Williams, 1968; Ljunghall & Hedstrand, 1975). Though several metabolic factors important in the formation of renal stones are known, the group of idiopathic stone formers still comprises a large proportion of the patient materials (Coe; Williams & Chisholm, 1976). It is therefore important to try to identify any further renal and metabolic factors that may be of significance in the pathogenesis of stones, in order to attempt to reduce the frequency of recurrences with adequate therapy.

The significance of prolonged and severe stone disease, as regards the renal function, has rarely been studied (Menne, Hillenbrand & Conrady, 1958; Edwards & Hodgkinson, 1965) and knowledge of the renal function in stone formers is scanty. Defects in the renal function may be considered to be of pathological significance in stone formation. In cases of prolonged and severe stone disease, it is also possible that impairments of the renal function may result from heavy stone formation.

The investigations presented here were made with the aim of studying the renal patho-physiology in relation to the nephrological-urological aspects of renal stone disease. Both glomerular filtration and primarily tubular functions have been investigated. The information that these studies have yielded will, it is hoped, provide improved guidance in the investigation, follow-up and selection of preventive therapy in patients with recurrent renal stones.

MATERIAL

Fifty-five patients with idiopathic, recurrent, renal stones (11 women and 44 men, aged between 22 and 67 years) were investigated. The majority of the patients had been referred from the Department of Surgery at the University Hospital in Uppsala or from hospitals in the region. Many patients had pre-

Table I. Type and number of operations in 27 patients with recurrent renal stones

	endoscopical extration (number of operations)	uretero-lithotomy	pyelo-lithotomy	nephro-lithotomy	resec-tion	Total
F (n = 6)	1	4	3[x)]	1	2	11
M (n = 21)	9	15	13[x)]	0	4	41
Total (n=27)	10	19	16	1	6	52

x) Analysis of stones was not performed in three cases

viously been investigated for their stone disease without any cause having been identified. No patient had cystinuria, primary hyperparathyroidism, chronic urinary tract infection, medullary sponge kidney or anatomical abnormality in the kidneys.

Operations: Twenty-seven patients (8 women and 19 men) had been operated upon. The frequency of operation was similar among the men and the women (Table I). Operations on ureters were more common (56 %) than operations on the kidney (44 %). The men had undergone endoscopic extraction and ureterolithotomy somewhat more often (58 %), compared with the women (45 %) (Table I).

Among the patients with mixed stones (calcium oxalate and calcium phosphate), resection of a renal pool had been performed in 23 % of all operations, compared with 4 % in patients with pure oxalate stones. Patients with pure phosphate stones had required operation in only two cases (Table II).

Stone composition: Stones from 31 patients were analysed. Women were found to have pure phosphate stones more often than men (38 %, compared with 17 %) (Table III). Men, on the other hand, were found to have pure oxalate stones more often (39 %, as against 25 %).

X-ray findings: Three patients had nephrocalcinosis and 35 patients had one or more demonstrable calculi in one or both kidneys.

Electrolytes in serum and urine: All the patients had normal serum calcium levels and normal serum alkaline phosphatases. Phosphate in the serum was

Table II. Types of operation in the different types of stones

	calcium oxalate (number of stones)	calcium phosphate (number of stones)	calcium oxalate + calcium phosphate (number of stones)
Endoscopical extraction	5	1	4
Uretero-lithotomy	12	1	6
Pyelo-lithotomy	7	0	6
Nephro-lithotomy	0	0	1
Resection	1	0	5
No operation	2	6	4
Total	27	8	26

Table III. Types of stones in 8 women and 23 men

	calcium oxalate (number of patients)	calcium phosphate (number of patients)	calcium oxalate + calcium phosphate (number of patients)
F	2	3	3
M	9	4	10
Total	11	7	13

in the lower normal range (0.7 - 0.8 mmol/l) in 14 patients. Uric acid in the serum was normal in all patients. One patient had hypokalaemia. Low plasma bicarbonate levels (20-22 mmol/l) were found in 10 patients. Twenty-two patients had hypercalciuria (>6 mmol/24 hours) and 27 patients had a somewhat low excretion of magnesium (<4 mmol/24 hours) in the urine.

Hormones: Aldosterone excretion in the urine was increased (>12 µg/24 hours) by between 13 and 31 µg/24 hours in 5 out of the 20 patients investigated. In one of these five patients, a raised serum renin level was found. The patients had no standardized sodium intake during the days before the urine collections.

METHODS

I. The investigation of renal function (I, III, IV)

1. Inulin- and PAH-clearances

Inulin- and PAH-clearances were investigated in 41 patients during hydropenia and water diuresis. Inulin and PAH were administered in priming doses of 0.03 g/kg body weight and 0.002 g/kg body weight respectively, followed by continuous infusions in which the doses were calculated according to the formula:

$$\frac{\text{Desired plasma concentration} \times \text{GFR} \times \text{RPF respectively} \times m^2 \text{ body surface}}{1.73},$$

where GFR is the estimated glomerular filtration and RPF the estimated plasma filtration. Two or three clearance periods of 10-30 minutes were performed during hydropenia and water diuresis respectively. Blood samples were taken in the middle of each clearance period. Urine was collected via an indwelling bladder catheter. Clearance was reported as the average of two or three clearance periods.

2. Concentration capacity

The renal concentration capacity was investigated by measuring the osmolality of the morning urine. In cases in which the value did not exceed 800 mosmol/kg, a pitressin test was commonly performed on hospitalized patients, The concentration capacity was investigated after a 19-hour thirst in 41 cases during the simultaneous measurement of inulin clearance and was reported as water re-absorption ($T^c_{H_2O}$). The normal values, according to Pitts (1970 a), are 1.5 - 2.5 ml/min.

3. Dilution capacity

The renal dilution capacity was investigated during the simultaneous measurement of inulin- and PAH-clearance in 41 patients by water loading. Water was administered orally in a dose of 2 % of the body weight initially, followed by 0.5 % of the body weight every 30 minutes. The dilution capacity was reported as free water clearance (C_{H_2O}). The normal values, according to Smith (1957), are 7-16 ml/min/100 ml GFR.

4. Acidification capacity

The renal acidification capacity was investigated in 53 patients by short ammonium chloride loading, during which ammonium chloride was administered in a dose of 150 mmol/m^2 body surface, in the form of either whole or crushed tablets. The acid-base status was investigated on arterialized capillary samples every hour (once before and 4-5 times after the administration of the tablets). Urine samples were collected every hour (once before and 5-6 times after the administration of the tablets). The urinary pH was analysed within 5-10 minutes. Urine for the investigation of titratable acid and ammonium ions was stored in a deep-freeze and analysed within 1 month. Phosphate was analysed on the day after the test (Backman, Danielson & Sohtell, 1976 a).

5. Bicarbonate loading

Twelve patients were investigated by the method of bicarbonate loading after prior acidification with ammonium chloride (100 mmol/m^2 body surface). The glomerular GFR was measured by inulin clearance. Bicarbonate was administered partly as a priming dose and partly by continuous infusions. The acid-base status was measured from arterialized capillary samples in the middle of each clearance period. Urine samples for the measurement of pH and P_{CO_2} were taken by a syringe in the urinary catheter.

II. The investigation of tubular proteinuria (V)

The excretion of β_2-microglobulin was investigated in 51 patients in the serum and in urine collected during a period of 24 hours. Degradation of the protein, which occurs in urine when the pH is below 5.5, was avoided by administering 10 g $NaHCO_3$ to the patients during the day on which the urine was collected.

In 15 patients, the excretion of β_2-microglobulin was investigated at increasing degrees of acidosis during ammonium chloride loading. Twelve patients were investigated during bicarbonate loading after acidification with ammonium chloride (100 mmol/m^2 body surface). The urinary excretion of β_2-microglobulin was investigated at increasing degrees of alkalosis. Urine from five healthy persons was also investigated after the intake of 15 g $NaHCO_3$.

III. Magnesium loading

In 27 patients, a somewhat low urinary excretion of magnesium (< 4 mmol/ 24 hours) and simultaneously a somewhat low serum magnesium (1.1 - 1.4 mmol/l) were observed. In an attempt to determine whether magnesium deficiency was present in these patients, a magnesium loading test was performed in 18 patients (Thorén, 1963). Magnesium was given intravenously in a dose of 10 mmol. The urinary excretion of magnesium was measured the day before and during the day of loading. It has been suggested that a low urinary excretion of magnesium after loading (< 80 % of the administered dose) indicates magnesium deficiency (Thorén).

Calculations

C_{osm} = osmolar clearance

U_{osm} = urine osmolality

P_{osm} = plasma osmolality

V = urine flow (ml/min

$T^c_{H_2O} = C_{osm}$

$C_{H_2O} = V - C_{osm}$

$C_{osm} = \frac{U_{osm} \times V}{P_{osm}}$

Bicarbonate in the urine during bicarbonate loading was calculated from the urinary pH and P_{CO_2} according to the Henderson-Hasselbalch equation:

$$\text{Urine pH} = pK + \log HCO_3^- - \log s\, P_{CO_2}$$

where pK is assumed to be $6.33 - 0.5 \sqrt{[Na^+] + [K^+]}$. s is the coefficient of solubility for CO_2 in urine (Slatopolsky, Hoffsten, Purkerson & Bricker, 1970).

Analytical methods

Calcium was examined with an Eppendorf flame photometer or atom-absorption spectrophotometer (Instrumentation Lab. 353, Mass., U.S.A.). Magnesium was measured with an atom-absorption spectrophotometer (Perkin Elmer 303). Inulin was investigated spectrophotometrically with diphenylamine (Hogeman, 1948). PAH was analysed with N-(1-naphthyl)ethylendiamine dihydrochloride (Smith, 1945). Sodium and potassium were analysed by a flame photometer (Model 343, Instrumentation Laboratory, Lexington, Mass., U.S.A.). Osmolality was investigated with a Knauer micro-osmometer

(M. Knauer, Berlin). The urinary pH was measured with a glass electrode G 297 and a calomel electrode K 497 at 38° C (Radiometer, Copenhagen). Titratable acid and ammonium ions were measured by the titration method (Jørgensen, 1957). Phosphate was analysed by Technicon Method N-4c (Technicon, New York, U.S.A.). The acid-base status was measured by an IL Automatic pH/blood-gas analyser (Model 313, Instrumentation Lab., Mass., U.S.A.) or Astrup's equilibration method (Radiometer, Copenhagen). P_{CO_2} in urine was measured by Astrup's equilibration method at 3 and 11 % respectively. β_2-microglobulin was analysed by radio-immuno-assay (Phadebas, AB Pharmacia Diagnostics, Uppsala, Sweden). Creatinine was measured by Technicon Method N-11b (Technicon, New York, U.S.A.).

RESULTS

The results from all the patients are presented in the appendix. Nine patients had more than one abnormality, exclusive of hypercalciuria. Isolated hypercalciuria existed in 25 % of the patients and in a further 25 % no abnormalities could be found (Table IV). Some defect was found in 72 % of the women and in 34 % of the men.

Inulin- and PAH-clearances (I)

Twenty-nine patients had an inulin clearance over 80 ml/min and 33 patients had a PAH clearance over 400 ml/min. Among the 12 patients who had an inulin clearance less than 80 ml/min were six patients over 50 years of age. Of the other 12 patients with reduced filtration, two had acidification defects and a further two had hypercalciuria, together with a long history of renal stones.

Acidification capacity (III, IV)

Acidification defects (RTA) of varying degrees of severity were found in 30% of the patients (64 % of the women and 20 % of the men) investigated. One patient had a complete tubular acidosis, two patients the incomplete type and seven patients had slight defects in the excretion of titratable acid, ammonium ions or phosphate. A delayed response to the ammonium chloride induced acidosis was found in six patients and was interpreted as a proximal acidification defect with wastage of bicarbonate. This diagnosis was confirmed by

Table IV . Diagnostic classification of 55 patients with recurrent renal stones

Isolated hypercalciuria	14
Acidification defects	8
Dilution defect	3
Tubular proteinuria	4
Suspected magnesium deficiency	3
Multiple abnormalities	9
No abnormality	14
Total	55

Table V. Type of stones in patients with renal acidification defects and hypercalciuria respectively

	calcium oxalate (number of patients)	calcium phosphate (number of patients)	calcium oxalate + calcium phosphate (number of patients)
acidification defect	3	5	2
hypercalciuria	4	2	7

an investigation with bicarbonate loading, in which the five patients investigated were found to have a low threshold for bicarbonate (18-22 mmol/l), a low T_m (1.8 - 2.1 mmol HCO_3^-/100 ml GFR) and an increased fractional bicarbonate excretion (C_{HCO_3}/C_{In}) (3-19 %). Two patients with distal acidification defects were, on bicarbonate loading, also seen to have incomplete tubular re-absorption of bicarbonate with reduced bicarbonate thresholds of 21-22 mmol/l.

Relation to the clinical picture: All the patients with the most severe form of stone disease, i.e. patients who had had many recurrences and several operations, had some form of acidification defect. Their medical histories were in several cases extremely long (up to 40 years) and in four cases the disease appeared to have worsened in recent years. The women had shorter histories, in four cases not longer than six years. Patients with acidification defects had every type of stone (Table V). Patients with pure phosphate stones had,

however, acidification defects in 70 % of the cases. Fifty per cent of the patients with acidification defects had been operated upon at some time, five of them more often. Slight acidification defects existed in five patients who had a smaller number of stones (less than five).

Simultaneous existence of hypercalciuria: Six patients (38 %) had simultaneous hypercalciuria, in two cases up to 15 mmol/24 hours, despite the lack of metabolic acidosis.

Relation to other abnormalities: Four patients with slight acidification defects had somewhat low plasma bicarbonate levels (<22.5 mmol/l). Three patients with RTA (in two the proximal type and in the other complete, renal tubular acidosis) had simultaneous dilution defects. Increased excretion of aldosterone in the urine was found in three patients, one of whom had an increased serum renin level. Two patients had a low urinary excretion of magnesium after loading.

Nephrocalcinosis was present in three patients, one of whom had a distal acidification defect and the other two proximal acidification defects.

Concentration capacity (I)

A slightly decreased concentration capacity was found in eight patients (685-780 mosmol/kg). The majority of these patients had long histories of renal stone. No correlation with acidification defects could be seen.

Dilution capacity (I)

There was a severely reduced ability to increase the urinary volume after water loading in seven patients (three men and four women). The mean value of free water clearance (C_{H_2O}) in these patients was -1.2 ml/min, compared with 10.1 ml/min in the other patients. The urinary osmolality and the urine volume were generally the same before and after water loading in these seven patients.

Relationship to clinical picture: Five patients had had frequent recurrences of stones. Four patients had been operated on one or more times by ureterolithotomy and/or pyelolithotomy. In two cases, it was necessary to perform a resection of the kidney. Two patients had only short histories of renal

stones.

Relationship to other abnormalities: All seven patients had low urinary excretions of magnesium and two had low excretions even after magnesium loading. Three patients had RTA, one of whom had hypercalciuria. Another patient had hypercalciuria as the only other abnormality. Two patients also had an increased aldosterone excretion in the urine, one of whom had an increased serum renin level as well.

The excretion of β_2-microglobulin in urine (V)

A total of 11 patients out of the 65 investigated were on some occasion found to have tubular proteinuria. The excretion of β_2-microglobulin was increased in four patients during normal conditions.

Four out of the five patients with distal acidification defects investigated had an increased excretion during acidosis and in two cases the excretion increased with increasing degrees of acidosis. Ten patients with normal acidification capacities showed no tubular proteinuria during provocation by acidosis.

Four out of eight patients with acidification defects increased their excretions of β_2-microglobulin during increasing degrees of alkalosis. One patient with a normal acidification capacity was found to have a greater excretion during alkalosis.

Relationship to the clinical picture: Eight out of 10 patients with increased excretions of β_2-microglobulin had had prolonged and severe stone disease. Four patients had been operated upon. Three patients with increased excretions during normal conditions also showed hypercalciuria.

Magnesium excretion after magnesium loading

Six patients (three men and three women) had low urinary excretions of magnesium after loading, of the order of 3.5 - 7.5 mmol/24 hours, which is lower than normal (Thorén).

Relationship to the clinical picture: Five patients had a more complicated type of stone disease, with recurrences relatively often. Three patients had

been operated upon. Stones from four patients were analysed. Mixed stones were found in three cases and a pure phosphate stone in one case.

Relationship to other abnormalities: Two patients also had simultaneous dilution defects, one of whom had RTA. Another patient was also found to have RTA. No patient had hypercalciuria.

GENERAL DISCUSSION

Renal involvement was frequently observed in this material of renal stone patients, in which one or more abnormalities of varying degrees of severity could be demonstrated in 49 % of the patients. A comparison with other materials is not possible, as such a broad investigation of renal function as is here described has not previously been reported. Many patients were included in this material who had had prolonged and severe stone disease, with many recurrences and many operations. This may explain the high frequency of abnormalities, as most of the defects were usually found in patients with severe stone disease. The women had defects significantly more often than the men. The women did not, however, have especially numerous stones, but despite this 55 % of them had been operated upon one or more times. This may indicate that larger stones are more common in women, which has also been shown in larger materials (Williams, 1968). The cause of this difference between the sexes has been ascribed to the higher frequency of chronic urinary tract infection in women (Williams). This was not the case in this material, and there was no evidence of chronic urinary tract infection in any of the patients. Recently, a relation has been indicated between stones that require operation and incomplete RTA (Froeling & Bijvoet, 1976).

Acidification defects

Distal acidification defects

In the distal type of renal tubular acidosis (RTA, type 1), there is an abnormality in the distal nephron, with an inability to excrete H^+ ions against a gradient (Morris, Sebastian & MacSherry, 1972). This results in an alkaline urine and a low excretion of titratable acid in both mild and more severe degrees of acidosis. In some cases, an incomplete RTA syndrome exists, in which a metabolic acidosis does not develop; this is considered to be due to a compensatory increase in ammonium ion excretion (Seldin & Wilson, 1966;

Morris et al). Usually, only the complete and incomplete types of renal tubular acidosis are considered. In this study, the concept of a distal acidification defect has been widened to include cases in which urine could be acidified to an acid pH below 5.0 but with a pathological excretion of titratable acid and/or ammonium ions. This is considered to be justified, as knowledge of the mechanism of the development of renal tubular acidosis is incomplete. As there is also a relation between RTA and the tendency to form renal stones, these slight defects ought to be taken into consideration and further studied. They may constitute a preliminary stage of RTA that is of pathogenetic importance in the stone formation in some patients. An early diagnosis of such slight defects would mean increased possibilities of giving adequate preventive therapy.

The pathogenesis of stone formation in the fully developed syndrome of renal tubular acidosis is considered to be hypercalciuria, low urinary excretion of citrate and alkaline urine. This will give rise to an increased risk of supersaturated urine. Both hypercalciuria and low citrate excretion are considered to be caused by the metabolic acidosis (Seldin & Wilson; Pitts, 1970 b) and to explain why certain patients develop osteomalacia (Seldin & Wilson).

However, hypercalciuria may also occur in the incomplete syndrome of RTA, which may mean that other factors than acidosis are of importance in the hypercalciuria. In four patients in this study, hypercalciuria was found, together with an incomplete and mild type of acidification defect. In two cases, the hypercalciuria was at a high level (15 mmol/24 hours). Important factors in this connection are therefore the role of hypercalciuria in the development of renal acidification defects and the extent to which cases with latent RTA are to be found amongst patients with hypercalciuria. Some cases of hypercalciuria in which a development to RTA has been followed have been described (Buckalew, MacCurdy, Ludwig, Chaykin & Elkington, 1963; Buckalew, Purvis, Shulman, Herndon & Rudman, 1974). Other slight variations in patients with hypercalciuria may also indicate a latent RTA. Thus, an incipient acidosis in cases with hypercalciuria was noted by Bradshaw & Boyce (1961). In another material, low urinary excretion of citrate was found (Nordin & Smith, 1963). Investigation of the urinary excretion of citrate seems to be important as low excretions were found in patients with the incomplete syndrome of familial RTA (Norman, MacCurdy, Cohn & Roth, 1975). These findings were interpreted as indications of a latent RTA.

In the material presented here, four patients among the cases with hypercalciuria had normal acidification capacities and somewhat low plasma bicarbonate levels (≤22 mmol/l). Such cases should be specially studied, in order to increase the knowledge of the importance of both the hypercalciuria and the RTA and the relation between them.

Proximal acidification defect

Cases with proximal, tubular acidosis (RTA, type 2) are relatively rarely described in the literature and renal stones have not been considered to be a characteristic finding (Morris et al; Donckerwolke, van Stekelenburg & Tiddens, 1970; York & Yendt, 1966; Soriano, Boichis, Stark & Edelmann, 1967). However, the majority of the cases reported have had a fully developed metabolic acidosis, making the diagnosis easier. A certain underdiagnosis of this acidification defect may be considered likely in cases with a slightly decreased re-absorption of bicarbonate, in which bicarbonate wastage has not been great enough to cause acidosis. No patients with proximal acidification defect in this study had developed metabolic acidosis and the diagnosis was possible only through ammonium chloride loading, after which the diagnosis could be verified by bicarbonate loading. During normal conditions, these patients have an alkaline urine, due to bicarbonate wastage. This would favour the development of renal stones, especially calcium phosphate stones, which were noted in several of our patients with this defect. Both pure phosphate stones and mixed stones of calcium oxalate and calcium phosphate were found in these patients. Hypercalciuria can also be found in connection with this acidification defect, especially during acidosis (Kazantzis, 1967). In the patients described here, hypercalciuria was either absent or was mild (7-9 mmol/24 hours), possibly due to the fact that the acidosis was mild.

In the patients described here, the proximal acidification defects may be primary. Two important differential diagnoses should, however, be considered in patients with a combination of renal stones and proximal acidification defect; namely, hyperparathyroidism, in which the defect is reversible after parathyroidectomy (Muldowney, Caroll, Donohoe & Freany, 1971), and chronic urinary tract infection. In the latter condition, an early defect in the acidification capacity has been observed, manifested in an incomplete tubular re-absorption of bicarbonate (Aperia, Berg & Broberger, 1974). These diagnoses could be excluded in our patients. However, in our patients it is also possible that the acidification defect may be secondary to the stone

disease.

Dilution defect

Convincing evidence, both from clinical and experimental work, illustrates the great importance of concentrated urine in the formation of renal stones (Vermeulen, Lyon, Ellis & Borden, 1967). A low urine volume thus considerably increases the risk of the precipitation of crystals. At low urine volume, the first calculi are also retained more easily at the papilla (Vermeulen et al). Therapeutically, this has been taken into consideration and a high fluid intake has been shown to reduce the frequency of stones considerably (Reinecke, Burehardt, Hallistratos, 1976; Blacklock, 1968).

In this material, a considerable number of cases with dilution defects was found (7 out of 41). A constantly low urine volume of less than one litre per 24 hours was noted in several patients with dilution defects and thought to be an important contributory cause of the very frequent formation of stones in these patients. However, it is difficult to judge the role played by a pure dilution defect, as most of the seven patients also had other defects that were probably of pathogenetic importance in the stone formation, such as RTA, low urinary excretion of magnesium and hypercalciuria. The causes of the dilution defects in these patients may be secondary to some other existing defect.

All patients with dilution defects had low urinary excretions of magnesium and in two cases a magnesium deficiency was suspected. It has been shown in animal experiments that sodium and fluid retention may occur, in cases of magnesium deficiency, possibly due to an altered transport of sodium and potassium through the cell membrane (Cantin, 1970; Whang & Welt, 1963; Elin, Armstrong & Singer, 1971). An intracellular sodium retention and a potassium reduction are obtained. The hyperaldosteronism that may occur in cases of magnesium deficiency (Whang & Welt; Cantin) and which was also found in two patients with suspected magnesium deficiency in this study may conceivably further accentuate the sodium retention. Clinically, water retention and oedema have been seen in some cases of magnesium deficiency (Haijamäe & MacDowall, 1972; Nielsen & Thorn, 1965). Suspected magnesium deficiency may thus be a possible cause of the dilution defect in two patients in this study.

In three other patients, a sodium wastage due to acidification defects, may be an explanation of the dilution defect. It is known that sodium deficiency may impair the dilution capacity, due to re-absorption of sodium in the proximal tubules (Wesson, 1969 a). In patients with RTA and dilution defects, the latter defect may thus be considered to be secondary to the acidification defect.

It is at present impossible to evaluate the importance of ADH as regards the occurrence of dilution defects, as investigations of ADH in these patients have not been conclusive.

Magnesium deficiency

The importance of magnesium deficiency in the development of renal stone formation has long been discussed on the basis of experimental studies (Hammarsten, 1956; Whang & Welt, 1963; Elin et al). Many workers have reported a low urinary excretion of magnesium in renal stone patients (Evans, Forbes, Sutton & Watson, 1967; Oreopoulos, Soyannwo & MacGeown, 1968; Hodgkinson, 1974). However, no reports of magnesium deficiency in renal stone patients have been published. Treatment with magnesium has, however, resulted in reduced rates of recurrence (Moore & Bunce, 1964; Melnick, Landes, Hoffman & Burch, 1971).

In this study, six patients had a suspected magnesium deficiency, as the urinary excretion of magnesium was low, even after magnesium loading. However, no clinical signs of magnesium deficiency were found. Symptomatic, primary, magnesium deficiency is probably rare and occurs chiefly in children (Haijamäe & MacDowall). In a case of primary magnesium deficiency, renal stone formation was reported (Paunier, Radde, Kooh, Conen & Fraser, 1968). The cause of the suspected magnesium deficiency in our patients is not clear. Isolated malabsorption of magnesium has been described (Paunier et al; Nordio, Donath, Macagno & Gatti, 1971; Haijamäe & Mac Dowall, 1972) and may have been present in the patients investigated here. The excretion of magnesium in urine was low and the serum magnesium was also somewhat reduced (1.1 - 1.4 mmol/l). There was no evidence of a deficient intake of magnesium. Further studies of magnesium metabolism would appear to be necessary, in order to clarify the mechanism of magnesium deficiency and its role in stone formation.

Magnesium is considered to be a "protective factor" and to increase the solubility of calcium oxalate (Smith , 1972; Desmars & Tawashi, 1973). The studies referred to here indicate that magnesium may also have another effect, namely, on the water excretion. Magnesium treatment of guinea pigs with magnesium deficiency has been found to double the volume of urine (Morris & O´Dell, 1969). Treatment with magnesium of patients with dilution defects should throw further light on the possible relation between dilution defects and magnesium deficiency.

Excretion of β_2-microglobulin in urine

Several signs of tubular involvement in renal stone patients were observed in these studies. Also patients with no other tubular dysfunctions could be shown to have a tubular affection in the form of tubular proteinuria. This appears to be an important finding, as it may indicate that there may be renal involvement later, as described in known tubular diseases. It has sometimes been possible to demonstrate tubular proteinuria at an early stage in familial tubular diseases, before other functional changes, for example, concentration and acidification defects, can be observed (Hall, Gaon, Griggs, Piscator, Popović, Vasilović & Zimonjic, 1967; Lindstedt & Lindstedt, 1973). In some of the patients described here who had tubular proteinuria, hypercalciuria was also found, which sometimes seemed to progress to a RTA (Buckalew et al). This justifies a special follow-up of these patients. In other patients in this study, tubular proteinuria could be provoked only by changes of the acid-base balance. This may mean that a tubular affection is demonstrable only during acidosis and alkalosis or that these patients´ renal tubules are specially susceptible to changes in acid-base balance.

The explanation of the appearance of tubular proteinuria in renal stone patients with otherwise normal renal functions is at present unclear. A careful follow-up and repeated studies are required,in order to illustrate the importance of these findings.

Glomerular filtration rate

In an earlier study on the renal function of renal stone patients, it was found that the glomerular filtration rate was somewhat low (Menne, Hillenbrand & Conrady, 1959). These investigators suggested that there was an associa-

tion between the lowered filtration rate and the stone disease and that the stone formation might even commence in the glomerulus. It has recently been shown in experimental studies that the first site of nucleation seems to be in the glomerulus and Bowman´s capsule, later followed by crystallization in the marrow and papilla (Bastian, Gebhardt & Harting, 1976). The reason for this was assumed to be a rise in the calcium level, with a supersaturation of the urine. Thus, in patients with a long history of renal stone disease and hypercalciuria, as in two of our patients with reduced GFR, the consequence may also be an affection of the glomerulus.

In another two of our patients, the reduced filtration rate may have been caused by their acidification defects. Even if the RTA by definition is a tubular disease, an affection of the glomerular filtration rate may sometimes be seen (Davies, 1967; Morris et al). The reduction of GFR has been found to be more pronounced in the complete form of RTA (Buckalew et al). One of our two patients with RTA and reduced GFR, also was found to have the complete type.

Most of the other patients in whom the GFR was reduced were more than 50 years of age and the cause in these cases may have been physiological, as it is known that GFR diminishes with age (Wesson, 1969 b). In our patients, the reduction was not more than could be accounted for by the age factor.

GENERAL SUMMARY

1. Renal function, primarily tubular but also glomerular function, was studied in 55 patients (11 women and 44 men) with idiopathic, recurrent, renal stones. A large number of the patients suffered from severe stone disease with rapid recurrences and had been subject to many operations. There were a large number of abnormalities among these patients, especially in the women.

2. One or more abnormalities were found in 49 % of the patients. Isolated hypercalciuria was found in 25.5 % and no abnormality in 25.5 %. However, all the patients were not studied in all respects.

3. The most common defect was loss of acidification capacity of varying degrees of severity. Impairment of the acidification capacity in the distal tubuli was found in 10 out of 53 patients and proximal acidification defect in six out of 53 patients. The latter defect was verified by investigations using bicarbonate loading.

4. Acidification defects were found in 64 % of the women and 20 % of the men.

5. Inability to dilute the urine after water loading was found in seven out of 41 patients. Dilution defects were often combined with other abnormalities, such as acidification defects and suspected magnesium deficiency.

6. Suspected magnesium deficiency was found in six out of 18 patients. The cause of this could not be ascertained.

7. Tubular proteinuria was noted in 11 out of 65 patients under normal conditions or during induced acidosis or alkalosis.

8. Glomerular filtration was slightly reduced in 12 out of 41 patients. Six of these were over 50 years of age.

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APPENDIX

A summary of all the investigations from the patients is made in this appendix.

The patients were divided into four groups according to how many stones they had suffered from.

"Duration" refers to the duration of the stone disease.

Table A I. Some clinical and laboratory findings in 22 patients who had suffered from 2-5 renal stones

Number of stones 2 - 5

Patient	Sex	Age	Duration years	Ca/u mmol/24 hours	Mg/u mmol/24 hours	Mg load mmol/24 hours	Dilution capacity	Concentration capacity	GFR ml/min	PAH ml/min	HCO_3^- mmol/l	Acid load	HCO_3^- load	β_2-microglobulin/urine	Stone composition
M.A.	F	23	6	3.5	3	0	-	820	82	545	25.5	-	0	-	
G.B.	F	36	2	8	3	-	+	680	98	680	22.5	Type 2	+	-	Hydroxyapatite
R.H.	F	51	28	4.5	4.5	0	0	750			23.5	-	0	-	
M.J.	F	67	6	6	-	0	0	990			27.0	Type 1	0	-	
K.L.	M	24	2	5	3.5	0	-	1 070	87	481	23.0	Type 1	0	-	
P.Å.	M	29	2	1.5	4	-	-	760	66	501	23.0	-	-	+	Ca oxalate + hydroxyapatite
P.P.	M	32	3	6	4	-	0	830			24.5	-	0	+	Ca phosphate
I.H.	M	33	5	0.5	4.0	0	0	845			24.5	-	0	-	
P.S.	M	34	6	11.5	7	0	0	782			25.0	-	0	-	Ca oxalate + hydroxyapatite
F.A.	M	36	11	11	5	0	0	925			24.5	-	0	-	Ca oxalate
B.H.	M	36	6	5.5	3.0	0	-	900	91	560		0	0		
H.W.	M	40	12	8	3	0	+	980	135	970	22.5	-	0	-	
KE.L.	M	41	1	5		0	0	890			24.0	-	0	-	
O.H.	M	42	5	9	3.5	0	-	960	89	515	22.5	Type 1	0	-	
G.S.	M	48	2	12	3	+	0	950			23.5	-	0	0	
M.K.	M	52	21	10	4	0	0	820			25.0	Type 1	0	-	
G.L.	M	52	2	5.5	2.5	0	-	940	75	317	24.5	-	0	-	
H.L.	M	52	22	9	3.5	-	-	795	84	512	24.5	-	0	-	
K.O.	M	52	4	4	3	0	-	1 000	89	466	23.0	Type 1	0	0	
B.I.	M	56	5	5	4	0	-	880	73	347	25.0	-	0	-	
S.B.	M	62	15	5	4	0	-	1 240	75	295	25.0	-	0	-	
E.J.	M	68	3	3	2	0	+	880	64	318	24.0	-	0	-	

0 = not performed
- = normal

Table A II. Some clinical and laboratory findings in 18 patients who had suffered from 6-10 renal stones

Number of stones 6 - 10

Patient	Sex	Age	Duration years	Ca/u mmol/24	Mg/u mmol/24	Mg load hours	Dilution capacity	Concentration capacity	GFR ml/min	PAH ml/min	HCO_3^- mmol/l	Acid load	HCO_3^- load	β_2-microglobulin/urine	Stone composition
E.W.	F	22	2	15	5.5	0	-	900	100	488	23.0	Type 1	-	-	Hydroxyapatite
I.M.	F	23	4	2.5	2.5	-	-	940	77	520	22.0	Type 1	+	+	Hydroxyapatite
C.P.	F	28	17	4.0	2.5	+	+	1 060	139	700	22.5	Type 2	+	-	Ca oxalate + phosphate
M.W.	F	35	4	4.0	2.5	+	-	800	83	429	21.5	Type 2	+	-	Ca oxalate + phosphate
I.A.	F	44	20	7.5	3.5	0	-	840	107	645	27.0	-	0	-	Ca oxalate + apatite
A.A.	F	35	12	5.0	1.9	+	0	860			22.5	-	0	0	Ca oxalate
S.E.	M	30	8	8	7	0	-	1 070	110	605	23.0	-	0	-	Ca oxalate + apatite
F.M.	M	33	17	8.5	5	0	-	880	77	400	21.0	-	0	+	Hydroxyapatite
O.S.	M	37	19	10	3	0	-	800	92	416	25.5	-	0	-	Ca oxalate + apatite
N.S.	M	41	9	8	4	0	-	860	93	508	22.0	0	0	-	
Å.P.	M	41	10	11	3	-	0				24.0	-	0	-	Ca oxalate
SE.M.	M	43	12	4.5	2	+	0	1 060			25.5	-	0	-	
B.S.	M	44	15	5	4	0	-	860	74	343	25.5	-	0	0	Ca oxalate
G.N.	M	46	14	8.5	5	0	-	850	111	499	25.5	-	0	-	Ca oxalate + phosphate
C.B.	M	48	17	9.5	6	0	-	920	94	568	22.0	-	0	-	
R.D.	M	50	3	5	5	0	-	1 050	78	490	22.0	-	0	0	
T.M.	M	52	30	4	2.5	0	-	880	69	378	23.5	-	0	-	
B.O.	M	56	18	11	5.5	-	0	700			23.0	-	0	-	Ca oxalate + ca phosphate

0 = not performed
- = normal
+ = pathological

Table A III. Some clinical and laboratory findings in 10 patients who had suffered from 11-25 renal stones

Number of stones 11 - 25

Patient	Sex	Age	Duration years	Ca/u	Mg/u	Mg load	Dilution capacity	Concentration capacity	GFR ml/min	PAH ml/min	HCO_3^- mmol/l	Acid load	HCO_3^- load	β_2-microglobulin/urine	Stone composition
				mmol/24 hours											
Y.S.	M	30	4	7.0	6.0	0	-	880	102	562	21.5	-	-	+	Ca oxalate
T.B.	M	36	17	2.2	2.3	+	+	810	93	495	25.0	-	0	-	Ca oxalate
S.D.	M	36	11	5	1.5	0	-	1 050	106	427	26.0	-	0	0	Ca oxalate + apatite
P.P.	M	39	8	5.5	5.5	0	-	880	88	518	29.0	-	0	-	Ca oxalate + apatite
Å.O.	M	40	21	7.0	2.5	-	-	890	79	431	25.0	-	-	-	Ca oxalate
B.S.	M	44	16	9	3	0	-	770	81	439	24.0	Type 2	0	0	Hydroxyapatite
A.B.	M	45	15	13	6.5	0	0	740			23.0	-	0	-	Ca oxalate + ca phosphate
H.A.	M	49	19	7.5	3.5	0	-	800	119	545	26.5	-	0	-	-
R.K.	M	56	29	5.5	6	0	-	760	82	302	25.0	-	0	0	Ca oxalate + hydroxyapatite
R.W.	M	58	37	5.5	3	0	+	880	82	386	25.0	-	0	-	Ca oxalate

0 = not performed
- = normal
+ = pathological

Table A IV. Some clinical and laboratory findings in 5 patients who had suffered from more than 25 renal stones

Number of stones > 25

Patient	Sex	Age	Duration years	Ca/u mmol/24 hours	Mg/u mmol/24 hours	Mg load mmol/24 hours	Dilution capacity	Concentration capacity	GFR ml/min	PAH ml/min	HCO_3^- mmol/l	Acid load	HCO_3^- load	β_2-microglobulin/urine	Stone composition
B.H.	F	30	6	5	3.5	-	+	850	76	481	17.5	Type 1	+	+	Ca oxalate
S.T.	M	34	9	5	4	0	-	840	98	662	26.0	Type 1	0	-	Ca oxalate
S.S.	M	48	27	7	5	-	-	810	84	416	23.0	Type 2	+	+	Ca oxalate
C.O.	M	50	40	5	4.5	-	-	810	107	658	22.0	Type 2	+	+	
H.P.	M	52	29	15	4.5	-	-	1 000	96	453	20.5	Type 1	0	+	Hydroxyapatite

0 = not performed
- = normal
+ = pathological

A SHORT DURATION RENAL ACIDIFICATION TEST

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ABSTRACT

As earlier investigations of renal acidification capacity were poorly standardized it was considered important to develop a clinically useful method. The ability of the kidneys to acidify urine was studied in 19 normal persons by short ammonium chloride loading. Ammonium chloride was given in a dose of 150 mmol/m^2 body surface in one of four different ways: whole tablets, crushed tablets, capsules and in solution. The degree of acidosis developed in each subject was measured in arterialized capillary blood samples. Urine was analysed for pH, titratable acid, ammonium ions (TA and NH_4^+) and phosphate. The excretion of TA, NH_4^+ and phosphate increased with the degree of acidosis in blood whilst urine pH decreased. The critical level appeared to lie around a base excess of -6 mmol/l below which all subjects could acidify their urine to a pH below 5.0. For clinical applications a frame of reference with tolerance limits is given for acidification capacity related to the degree of acidosis expressed as base excess.

Key words: Renal acidification; hydrogen excretion; titratable acid; ammonium ion excretion

INTRODUCTION

For a more complete evaluation of the kidney function it is important to test the acidification capacity not only in renal diseases but also in other disorders where renal tubular functions may be affected such as liver diseases, hyperparathyroidism and hypergammaglobulinaemia. In mild forms of acidification defects there is no systemic acidosis and the only way to find the defect is by an acid loading test.

In the fully developed syndrome of renal tubular acidosis the diagnosis is easily established from the metabolic acidosis and alkaline urine. This syndrome is often associated with frequent renal stone formation (Seldin & Wilson, 1966). Therefore it seems important that patients with idiopathic renal stone should be investigated with an acid loading test as even mild defects in acidification capacity can be of pathogenetic importance for stone formation (Buckalew, MacCurdy, Ludwig, Chaykin & Elkinton, 1968).

Various accounts of the method by which these tests should be performed (administration, sampling, etc.) can be found in the literature (Wrong & Davies, 1959; Morris & Fudenberg, 1967; Edelman, Boichis, Soriano & Stark, 1967; Tannen, 1971; Adler, Lindeman, Yiengst, Beard & Shock, 1968). Often, however, a sufficient acidosis was not achieved which is important in evaluating results from patients, especially in diseases affecting the proximal tubules. In proximal acidification defects urine pH decreases to acid levels only at a higher degree of acidosis (Morris, Sebastian & Mac Sherry, 1972). This report serves to evaluate several factors e.g. mode of administration and degree of acidosis achieved in normal subjects in an attempt to develop a suitable, clinically useful method in which the acidification of the urine could be related to the degree of acidosis.

MATERIAL

The studies were performed on 19 healthy persons who gave no evidence of renal disease or any other disease affecting the kidney function. There were ten men and nine women aged 23-50 years.

As it was desirable to applicate the test in clinical use a frame of reference was calculated. For this purpose a sufficient number of observations was

necessary. It seemed justified to include normal acidification results from 39 renal stone formers. Seven of these patients are also described in another report (Backman, Danielson & Sohtell, 1976). There were thirty-one men and eight women aged 23-53 years. These patients were found to have a normal acidification capacity as compared with the 19 healthy persons. One patient had suffered from about 20 stones and the others from 1-10 stones. There was no history of other renal disease or hypertension in these patients. They had normal concentration of creatinine and calcium in serum and concentration of morning urine exceeded 700 mOsmol/kg. Only one patient had a mild hypercalciuria, 7.5 mmol/24 hours.

METHODS

In the morning after the induction of a diuresis with 200-400 ml water, ammonium chloride was given in a dose of 150 mmol/m^2 body surface in one of four ways. The subjects ate normally and normal physical activity was permitted. Approximately one hundred ml of water per hour were consumed throughout the experiment.

1. Whole tablets were given to seven persons. The tablets were taken over an one hour period together with breakfast.

2. Crushed tablets were administered to another seven persons. The tablets were crushed in order to facilitate absorption as in some cases in which whole tablets had been given no acidosis was obtained.

3. Capsules were given to five subjects. Capsules were used to reduce gastrointestinal reactions.

4. Solution about 50 ml 4M was given to four fasting subjects who remained fasting throughout the experiment. These four subjects also took whole tablets.

The ammonium chloride was administered to 24 of the renal stone patients as whole tablets, whilst the remaining 15 were given crushed tablets.

Sampling

Arterialized blood capillary samples for the analysis of acid-base status were taken after warming the hand, before ammonium chloride was administered and in the middle of each urine collection period, that is four to nine times depending on the mode of administration. A urine sample was collected prior to ammonium chloride intake and every hour thereafter. The urine samples were collected in a test-tube which was immediately corked. Urinary pH was measured within 15 minutes. The urine samples were kept without adding any preservative in a deep freeze and titratable acid and ammonium ions were analysed within a month. Phosphate was analysed the day after the acid loading test.

Arterial blood samples should be taken in the assessment of acid-base status. Arterial punction was too sophisticated a procedure to be used in a test in which repeated samples must be taken. Arterialized capillary samples do not differ too much from arterial samples (Siggaard-Andersen, 1974) making them satisfactory in this test.

Storage of samples

A storage test was performed on urine samples to be analysed for titratable acid and ammonium ions. Morning urine from two subjects were divided into five portions and kept at $+4^{\circ}$ C and at -20° C respectively. Double samples were titrated immediately, after 24 hours, 48 hours, 5 days and after one week. Samples from three patients were also titrated after one, two and four weeks.

Statistical methods

For clinical evaluation a frame of reference was calculated where the results from 39 stone formers were added to those from the 19 normal subjects. The acidification of each subject was plotted against the metabolic acidosis expressed as base excess (BE) on the abscissa and the urine parameters (pH, TA, NH_4^+ and phosphate) on the ordinate. The variations in urine variables were described with limits within which a new case with a certain probability would lie. The limits are known as prediction or tolerance limits and have been arrived at thus: The abscissa was divided into intervals of 1 mmol/l, at the extremes two and four intervals respectively have been

combined because of the paucity of observations in these regions. No subject had more than one observation in each interval. Means and standard deviations of the urine parameters were calculated for each interval and tolerance limits were calculated according to the formula:

$$\text{tolerance limit} = \bar{x} \pm t_{(n-1)} SD \sqrt{1 + \frac{1}{n}}$$

where $t_{(n-1)}$ is the upper $(1 - \frac{\alpha}{2})$ 100 % point of the Student's t-distribution with n - 1 degrees of freedom. These limits include $(1 - \alpha)$ of the population on an average and the limits were given for $\alpha = 0.05$ unilaterally for urine pH, titratable acid and phosphate. Both upper and lower limits were given for ammonium ion excretion.

Student's t-test was used for calculation of significance.

Analytical methods

1. Blood: The capillary samples were kept in iced water and acid-base status was analysed either with and IL model 313 Automatic pH/Blood Gas Analyser (Instrumentation Lab. Inc. Mass) or Astrup's equilibration method (Radiometer, Copenhagen). The actual values for total CO_2, HCO_3^- and base excess were obtained by plotting the values obtained on a Siggaard-Andersen curve nomogram (Radiometer, Copenhagen).

2. Urine: Urine pH was measured with glass electrode G 297 and Kalomel electrode K 397 at 38° C (Radiometer, Copenhagen). Titration according to Jørgensen (1957) was used for the measurement of titratable acid and ammonium ions. Phosphate was measured spectrophotometrically with Technicon method n-4c (Technicon, New York).

RESULTS

Storage of samples: There were no differences in results from samples stored for different periods at the same temperature (Fig. 1, Table I). The method of storage of urine samples showed that these could be stored in a refrigerator for up to at least a week, in a deep freeze for up to at least a month.

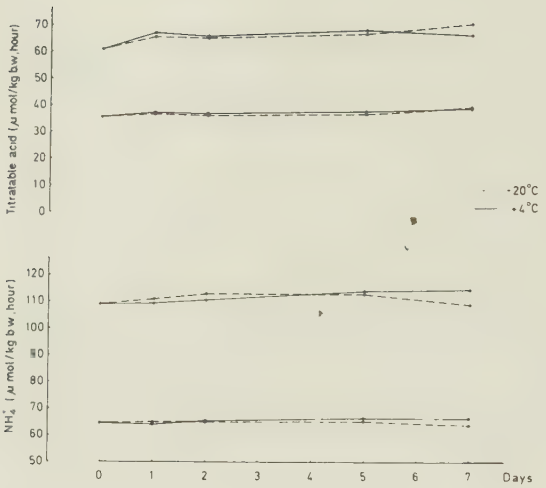

Fig. 1. Concentration of TA and NH_4^+ after various intervals of storage of samples at $+4^{\circ}$ C and -20° C respectively.

Table I. Concentration of TA and NH_4^+ after various intervals of storage of samples at -20° C

		Sample 1		Sample 2		Sample 3	
Patient	Time	TA	NH_4^+	TA	NH_4^+	TA	NH_4^+
K.J.	1 week	4	22	7	20	6	22
	2 weeks	3	21	4	18	9	23
	4 weeks	4	26	5	21	9	26
S.T.	1 week	1	11	3	17	7	20
	2 weeks	-3	15	0	14	5	21
	4 weeks	-3	15	3	17	7	25
H.A.J.	1 week	10	45	13	25	8	28
	2 weeks	9	40	14	24	6	27
	4 weeks	12	44	17	25	8	29

TA and NH_4^+ in mmol/l

Table II. The time taken for development and degree of acidosis achieved after ammonium chloride loading in 19 normal subjects

	Blood		
	Base excess mmol/l	Total CO_2 mmol/l	Time to develop max. acidosis, hours
	1. Whole tablets (n = 7)		
Mean:	-8.4	13.4	
SD:	±3.0	±4.3	
Range:	-3.8 - -12.5	12.0 - 20.0	6 - 8
	2. Crushed tablets (n = 7)		
Mean:	-10.8	13.5	
SD:	±1.7	± 1.3	
Range:	-8.0 - -12.3	12.2 - 16.0	1.5 - 3.5
	3. Capsules (n = 5)		
Mean:	-6.4	16.8	
SD:	±1.7	±1.7	
Range:	-4.0 - -8.8	14.5 - 19.1	6 - 9.5
	4. Solution (n = 4)		
Mean:	-11.5	14.1	
SD:	±2.9	±2.7	
Range:	-7.3 - -14.1	11.2 - 17.8	2.5

Metabolic acidosis achieved after loading according to administration: All 19 normal subjects developed an acidosis (range for base excess -4 to -14 mmol/l) most marked in groups 2 (crushed tablets) and 3 (solution), where also the most rapid acidification was observed (Table II). Administration of ammonium chloride in capsule form (group 3) gave only a moderate acidosis that in two cases took a long time (8-9 hours) to develop. Also when whole tablets (group 1) were given it took several hours for an acidosis to develop in some cases. The difference between group 2 and 3 for maximal base excess was significant ($p < 0.001$) and also between group 3 and 4 ($p < 0.02$).

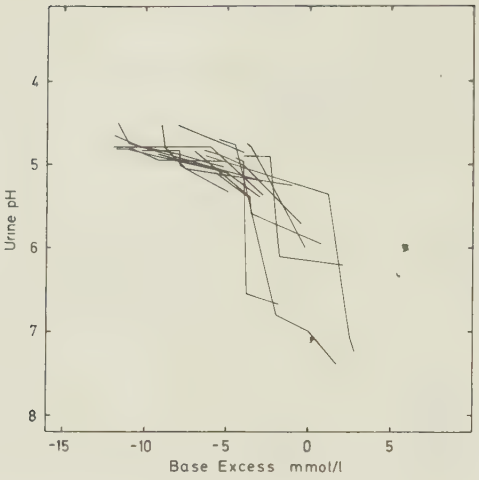

Fig. 2. Urine pH related to the metabolic acidosis in 19 normal subjects

Table III. Maximal H^+ (pH, TA and NH_4^+) and phosphate excretion after ammonium chloride loading in 19 healthy subjects

	Urine			
	pH	TA	NH_4^+	Phosphate x)
	1. Whole tablets (n = 7)			
Mean:	4.75	32	64	33
SD:	±0.13	±5	±17	9
Range:	4.50 - 4.9	25 - 41	48 - 90	25 - 50
	2. Crushed tablets (n = 7)			
Mean:	4.72	31	76	31
SD:	±0.12	10	12	14
Range:	4.53 - 4.83	20 - 51	68 - 103	15 - 59
	3. Capsules (n = 5)			
Mean:	4.75	28	66	26
SD:	±0.18	13	16	7
Range:	4.52 - 4.95	16 - 49	45 - 87	17 - 36
	4. Solution (n = 4)			
Mean:	-	29	57	27
SD:	-	3	13	8
Range:	-	26 - 34	48 - 67	19 - 38

x) TA, NH_4^+ and phosphate in µmol/hour and kg b.w.

Table IV. Maximal H^+ (pH, TA and NH_4^+) and phosphate excretion after ammonium chloride loading in 39 renal stone formers

	Urine			
	pH	TA	NH_4^+	Phosphate x)
Mean:	4.80	29	62	30
SD:	±0.21	±6	±14	±7
Range:	4.48 - 5.02	14 - 47	35 - 93	17 - 48

x) TA, NH_4^+ and phosphate in µmol/hour and kg b.w.

Side effects due to the ammonium chloride: Gastrointestinal symptoms such as nausea, vomiting and diarrhoea occurred in a few instances in group 1, 2 and 3. All the subjects in group 4 experienced violent nausea and one vomited, but, despite vomiting, a normal acidification was obtained.

Urinary acidification in relation to acidosis

All the 19 normal subjects acidified their urine to a pH lower than 5.0 when base excess reached -6 mmol/l (Fig. 2). A simultaneous increase in titratable acid and ammonium ions was obtained. Maximal values for pH, TA, NH_4^+ and phosphate are given in Table III. There were no differences between the groups in the excretion of TA. In the excretion of NH_4^+ there was a slight difference only between group 2 and group 4 ($0.02 < p < 0.05$).

Frames of reference for normal urinary acidification

Figure 2 shows the urine pH in relation to BE for the 19 normal subjects. In order to calculate a frame of reference with a sufficient number of observations in each BE interval, 39 renal stone formers were included. All the patients were found to have a normal acidification capacity compared to the 19 normal subjects. The following criteria had to be fulfilled: urine pH had to be within the outer limits given in Figure 2. Maximal values for TA, NH_4^+ and phosphate in each individual had to be within the ranges given in Table III. Means and standard deviations for maximal values of urine pH, TA, NH_4^+ and phosphate are shown in Table IV. There were no differences between the 39 patients and the normal subjects.

Table V shows the means, standard deviations and tolerance limits for urine pH, TA, NH_4^+ and phosphate for the different intervals of base excess. Figure 3-6 show the different urine parameters with means and tolerance limits related to the BE.

DISCUSSION

The introduction of a short ammonium chloride loading test in the investigation of renal acidification capacity in 1959 by Wrong & Davies was a great advance and showed that the short loading gave similar information to that obtained after loading over several days. However, their material (Wrong & Davies) and later studies of normal adults (Morris & Fudenberg; Adler et al) lack data on the degree of acidity in urine in relation to the degree of acidosis obtained. Edelman et al found no relationship between acidosis and acidification of urine in a paediatric material. Only one blood sample was, however, taken after loading and the result of the urinary analyses was given as the mean of three urine collections. Tannen (1970) was also unable to demonstrate any such relationship either. However, in his study only a very mild acidosis was achieved.

The mode of administration of ammonium chloride has generally been capsules (Wrong & Davies; Tannen) or tablets (Morris & Fudenberg). The dose given has usually been 0.1 g/kg body weight (75 mmol/m^2 body surface) (Morris & Fudenberg; Wrong & Davies). This causes only a mild acidosis, if it achieves one at all (Wrong & Davies; Adler et al). Acid-base status should always be examined to ensure that an acidosis is achieved as it was found that both capsules and tablets failed to be absorbed in a couple of subjects. Edelman et al tried two different dosages and found that a higher dose (150 mmol/m^2 body surface) gave a greater acidosis and facilitated appraisement of acidification capacity. The achievement of a sufficient acidosis appears to be clinically important to rule out proximal tubular acidosis where urine pH decreases to acid levels only at a higher degree of acidosis (Morris, Sebastian & Mac Sherry, 1972).

In this study 150 mmol/m^2 body surface were administered and the degree of acidosis achieved was dependent on the mode of administration i.e. crushed tablets and solution gave the highest degree of acidosis. It is important that a test for clinical use as well as being effective, i.e. causing a highgrade

Table V. Means, standard deviations and tolerance limits of the urinary pH, TA, NH_4^+ and phosphate for each BE-interval during ammonium chloride loading in 19 healthy subjects and 39 renal stone formers

	Base Excess (mmol/l)												
Urine pH	-14 to -11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	0 to +1	+2 to +3
n	17	16	12	21	22	22	18	28	14	16	14	18	11
Mean	4.74	4.70	4.79	4.82	4.82	4.93	5.05	5.07	5.27	5.63	5.99	6.06	6.32
S.D.	± 0.18	±0.17	±0.13	±0.15	±0.13	±0.19	±0.33	±0.34	±0.43	±0.78	±0.71	±0.72	±0.88
95 %	5.06	5.03	5.04	5.09	5.05	5.27	5.64	5.66	6.06	7.08	7.29	7.35	7.98
TA µMol/kg b.w., hour													
n	17	19	12	31	28	26	28	35	18	20	16	22	-11
Mean	29	26	26	24	23	24	21	19	12	12	4	1	-1
S.D.	± 9	± 7	±10	± 8	± 8	±11	± 9	±12	±10	±14	±12	± 9	±12
95 %	13	13	8	11	10	6	5	-2	-6	-13	-18	-15	-25
NH_4^+ µMol/kg b.w., hour													
n	17	19	12	31	28	26	28	35	18	20	17	22	11
Mean	75	61	61	60	57	55	57	51	37	42	31	26	28
S.D.	±13	±17	±21	±20	±18	±21	±19	±18	±17	±20	±16	±13	±12
95 %	48-103	24-98	13-109	18-102	19-95	11-99	17-97	14-88	0-74	0-85	0-66	0-54	0-56
Phosphate µmol/kg,bw.,h.													
n	17	19	12	31	24	26	28	35	18	19	14	17	9
Mean	28	28	29	25	27	24	22	18	15	13	11	8	9
S.D.	±11	± 9	± 8	± 9	±12	±11	±10	±10	± 8	± 7	± 5	± 4	± 6
95 %	10	12	14	9	6	5	5	1	1	1	2	1	0

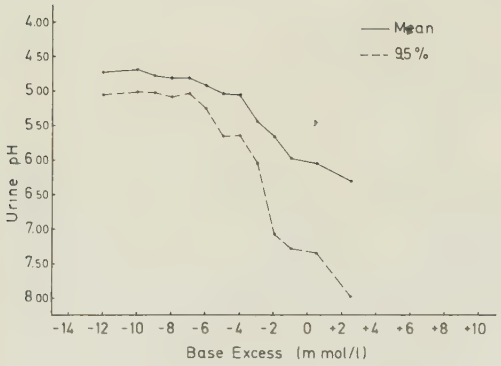

Fig. 3. The change in urine pH expressed as the mean of each BE-interval and the lower 95 % tolerance limit related to the degree of acidosis in 19 normal subjects and 39 renal stone formers

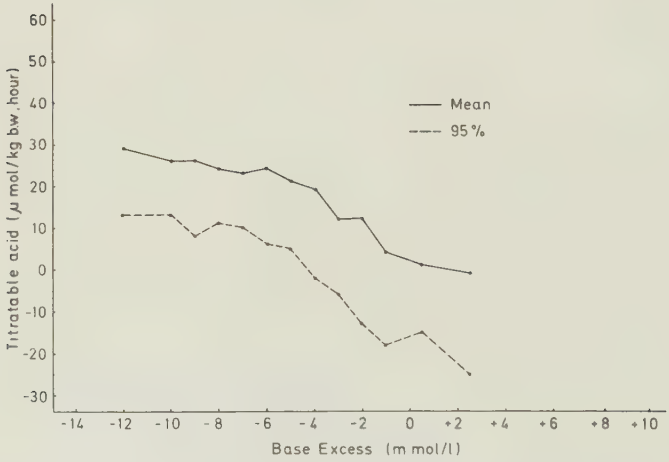

Fig. 4. Excretion of TA expressed as the mean of each BE-interval and the lower 95 % tolerance limit related to the degree of acidosis

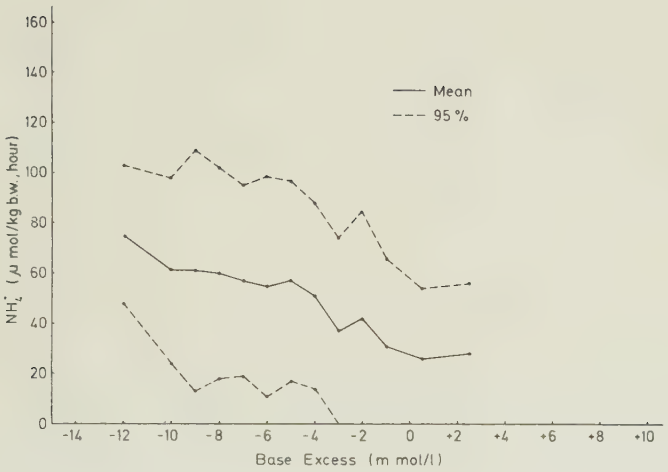

Fig. 5. Excretion of NH_4^+ expressed as the mean of each BE-interval and the lower 95 % tolerance limit related to the degree of acidosis

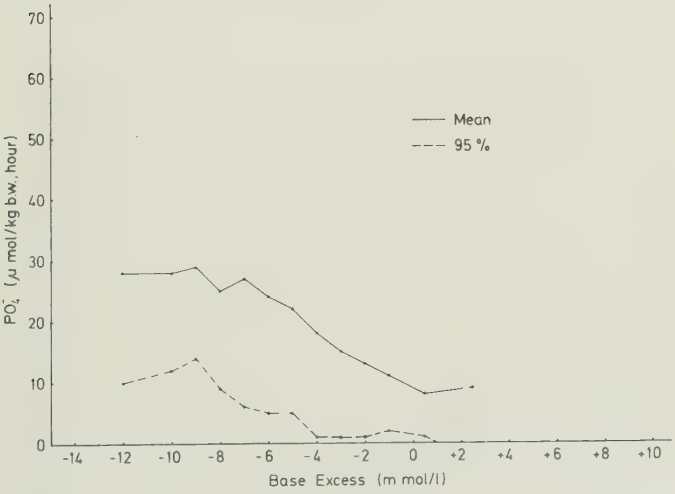

Fig. 6. Excretion of phosphate expressed as the mean of each BE-interval and the lower 95 % tolerance limit related to the degree of acidosis

acidosis gives as few side-effects as possible and takes as short a time as possible to perform. Crushed tablets have, in this study, been shown to fulfill these criteria. As the acidosis achieved in our study was high we could show that the renal excretion of H^+ as titratable acid and NH_4^+ increased with the degree of acidosis contrary to some other reports (Edelman et al; Tannen). The critical range for normal acidification of urine appeared to lie between a base excess of -3 to -6 mmol/l, below which level all subjects had acidified their urine to a pH lower than 5.0.

To permit evaluation of patient material it is necessary to fix limits for the normal variations, given in this investigation as tolerance limits. Other studies often lack a normal range (Tannen; Adler et al; Morris & Fudenberg) or this is given as regression lines for TA, NH_4^+ and urine pH respectively (Wrong & Davies). Only the lower limits for urine pH and TA were given in this investigation as only deviations in this direction (i.e. low excretion of TA, increased urine pH) are of interest. That will say only an impaired excretion of H^+ ions will be suggestive of acidification defects. In using a frame of reference with tolerance limits given it will also be possible to diagnose mild acidification defects which may be of clinical importance.

This material is too small to allow an age-grouping which otherwise would have been desirable as older persons have been shown to have a lower acidification (Adler et al).

In clinical evaluation of acidification capacity both reduced ammonium ion excretion - primarily in association with reduction in renal parenchyma (Pitts, 1973) - and increased ammonium ion excretion - as in renal tubular acidosis - (Seldin & Wilson, 1966) can be seen. The variation in ammonium ion excretion in the present study was large but was of the same magnitude as in other studies of normals (Morris & Fudenberg; Wrong & Davies). It cannot, however, be considered to be maximal as it is known that ammonium ion excretion is significantly greater in chronic metabolic acidosis than in acute acidosis (Pitts, 1948). This is probably due to the fact that H^+ concentration in the renal tubules in acute acidosis is of major importance for the conversion of NH_3 to NH_4^+ (Rector, Seldin & Copenhaver, 1955) while no activation of transport mechanisms or enzymes has time to commence to convert glutamine to NH_4^+ as occurs in chronic acidosis (Rector et al; Pitts, 1973; Goldstein, 1975).

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URINE ACIDIFICATION CAPACITY IN RENAL STONE FORMERS

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Scand J Urol Nephrol, Suppl. 35, 49, 1976.

ABSTRACT

A short ammonium chloride loading test was used in the investigation of renal acidification capacity of 51 patients with idiopathic recurrent renal stones containing calcium. Many of these patients had suffered from prolonged and severe stone disease. In 31 % of the patients an impaired acidification with varying degrees of severity was found. Eight patients had defects suggesting distal renal tubular acidosis (RTA). One case with the complete and two cases with the incomplete form of RTA were found. A further five patients, who were all able to acidify their urine to an acid pH, had milder defects in the excretion of titratable acid and/or ammonium ions which may indicate an early stage of distal tubular acidosis. Six patients were suspected of having a proximal acidification defect, as they showed delayed responses to the ammonium chloride induced acidosis.

Key words: Hydrogen excretion; renal stone disease; renal acidification; renal tubular acidosis; calcium excretion

INTRODUCTION

Renal tubular acidosis is considered to be a rare disease in its fully developed, complete form. It is a serious condition as it is often combined with nephrocalcinosis and osteomalacia may develop. Renal insufficiency may be the eventual result (Buckalew, Purvis, Shulman, Herndon & Rudman, 1974). A milder, incomplete form also exists, first described by Wrong & Davies (1959), in which metabolic acidosis does not occur but in which the acidification of the urine is defective. Hypercalciuria, renal stones and nephrocalcinosis are often present and without treatment this form may progress to the fully developed syndrome (Buckalew, MacCurdy, Ludwig, Chaykin & Elkinton, 1968; György & Edwards, 1968; Wrong & Davies). The incomplete form can only be diagnosed after ammonium chloride loading. As progressive stone formation and nephrocalcinosis may be hindered by therapy the diagnosis should be made as early as possible. The major sign of the incomplete form is renal stones. Patients with idiopathic renal calculi thus may have acidification defects that are of importance in the stone formation.

MATERIAL

Fifty-one patients, 40 men and 11 women aged 23-68 years, were investigated. Only patients with idiopathic stone disease were investigated and patients with conditions known to cause stones, i.e. hyperparathyroidism, chronic pyelonephritis, medullary sponge kidney or anatomical abnormalities were excluded. Many of the patients had a severe stone disease and had been investigated earlier to find the cause of the stone formation. Seven of the patients described in an earlier report were found to have a normal acidification capacity (Backman, Danielson & Sohtell, 1976).

METHODS

Short ammonium chloride loading was used. The patients took crushed ammonium chloride tablets in a dose of 150 mmol/m^2 body surface. The acid-base status was measured from arterialized capillary specimens before tablet administration, as well as in the middle of each urine collection period (4-6 times). Urine samples were collected each hour and the pH was measured immediately. Titratable acid (TA), ammonium ions and phosphate of the urine

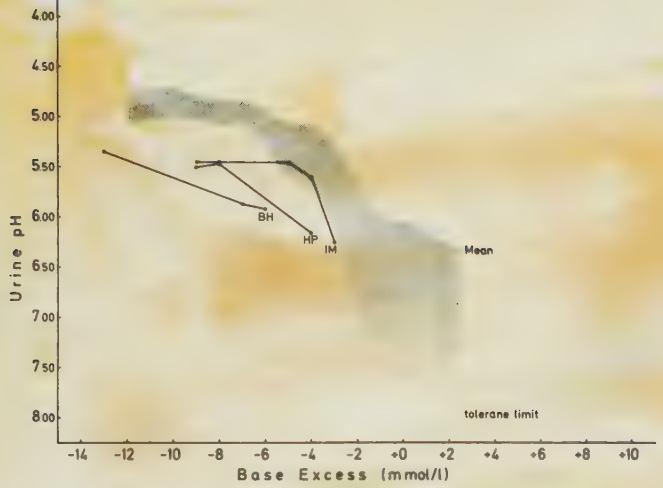

Fig. 1. The pH in the urine related to the BE in three patients with distal acidification defects

were analysed as described previously (Backman et al).

RESULTS

A frame of reference, giving the tolerance limits for normal acidification capacity was described in a previous report (Backman et al). In relation to this frame of reference, 35 patients were found to have a normal acidification capacity.

The urine pH did not decrease below 5.4 - 5.5 in three patients, one man (H.P.) and two women (B.H. & I.M.) indicating a distal acidification defect (Fig. 1). One woman (B.H.) had the complete form with a metabolic acidosis even under normal conditions with a base excess (BE) of -6 mmol/l. She was found to have a low excretion of TA but could increase this to normal levels with maximal acidosis (Fig. 2). The excretion of NH_4^+ was normal in her normal condition and increased to over the normal at maximal acidosis (Fig. 3). The other two patients had a mild metabolic acidosis under normal conditions and were considered to have the incomplete form. Their excretion of TA was normal and increased only slightly during acidification (Fig. 2). All three had a normal phosphate excretion in relation to our frame of reference (Backman et al).

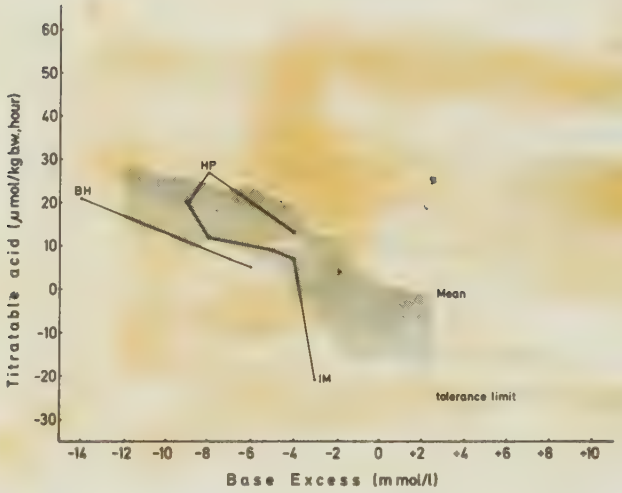

Fig. 2. Excretion of titratable acid in urine related to the degree of metabolic acidosis (BE) in three patients with distal acidification defects

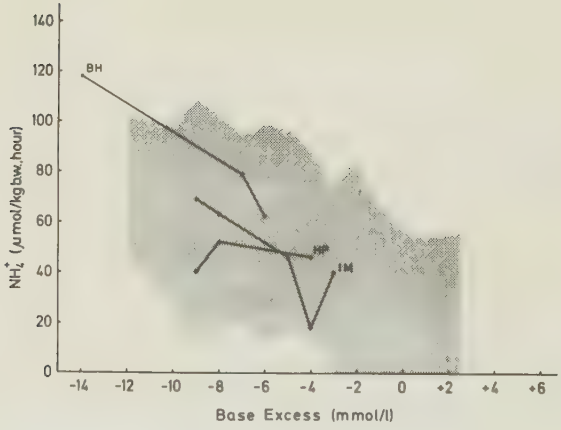

Fig. 3. Excretion of NH_4^+ in urine related to the BE in three patients with distal acidification defects

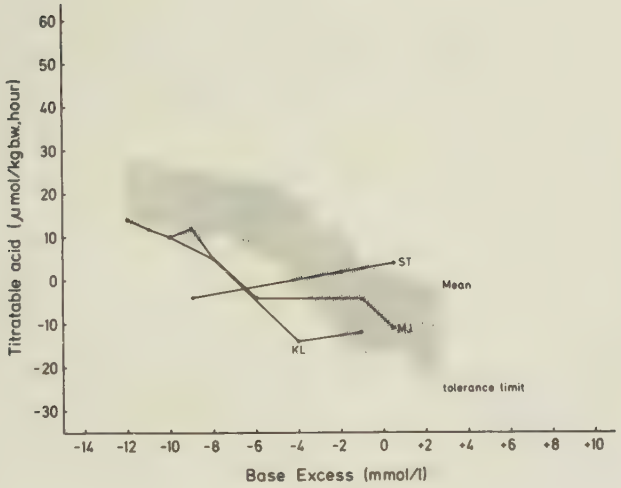

Fig. 4. Excretion of titratable acid in urine related to the BE in three patients with mild acidification defects

A further five patients, who could reduce their urinary pH to normal acidic levels had milder defects in the form of low TA excretion (Fig. 4) and/or increased NH_4^+ excretion (Fig. 5) with increasing degree of acidosis. Phosphate excretion was normal and increased with increasing degree of acidosis.

Two other patients had abnormally low phosphate excretion (4 µmol/kg b.w. hour), which did not increase during acidification, but, despite this, they had normal TA excretions.

The urinary pH sank to a normal acid level in a further six patients but at a higher degree of acidosis than normal (Fig. 6). This may indicate proximal tubular acidosis.

Table I and II show some clinical and laboratory findings for patients with and without acidification defects respectively.

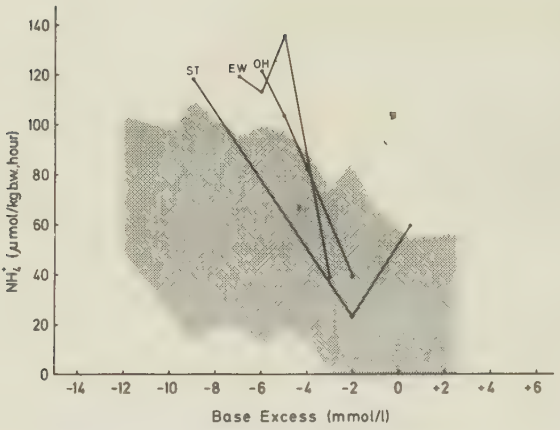

Fig. 5. Excretion of NH_4^+ in urine related to the BE in three patients with mild acidification defects

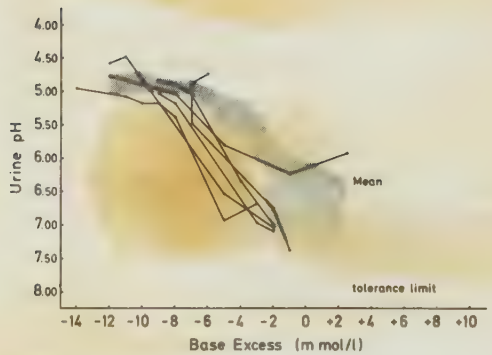

Fig. 6. The pH in the urine related to the BE in six patients with suspected, proximal, tubular acidosis

Table I. Clinical and laboratory findings in 16 patients with different types and degrees of acidification defects

Patient	Sex	Age	Age at onset	Number of stones	Stone composition	Nephro-calci-nosis	GFR ml/min	Concentration capacity mOsmol/kg	HCO_3^-/s mmol/l	Ca/s mmol/l	Ca/u mmol/24 h	Phosphate/s mmol/l
RTA type 1												
B.H.	F	29	24	> 25	Ca oxalate	+	73	800	17.5	2.4	5	1.1
I.M.	F	22	19	7	Hydroxyapatite	-	78	940	21	2.3	3	1.0
H.P.	M	51	23	> 100	Hydroxyapatite	-	96	1 000	20.5	2.3	15	1.0
Mild acidification defect												
S.T.	M	33	25	> 25	Ca oxalate	-	98	810	26	2.4	6	0.9
E.W.	F	21	20	7	Hydroxyapatite	+	95	900	22	2.3	16	1.1
O.H.	M	41	37	3	-	-	89	940	22	2.3	10.5	0.9
K.L.	M	23	22	3	Ca oxalate	-	87	1 070	23	2.5	5.5	1.0
M.J.	F	67	61	4	-	-	-	990	25	2.5	5.5	1.0
RTA type 2												
M.W.	F	34	31	6	Ca oxalate + Ca apatite	-	83	1 090	21.5	2.4	3	1.1
G.B.	F	35	34	2	Hydroxyapatite	-	98	690	22.5	2.5	8	0.8
C.P.	F	27	11	8	Ca oxalate + phosphate	-	139	1 000	22.5	2.4	6.5	0.8
C.O.	M	49	10	100	-	+	96	1 242	22	2.4	4.5	0.8
S.S.	M	47	21	Several 100	Ca oxalate	-	81	810	22	2.3	7	0.8
B.G.	M	42	26	15	Hydroxyapatite	-	81	1 070	25	2.5	11	0.9
Low urine excretion of phosphate												
K.O.	M	50	48	2	-	-	89	980	22.5	2.5	4	0.8
M.K.	M	51	31	4	-	-	-	820	24	2.4	10	0.8

Table II. Clinical and laboratory findings in 35 patients with no RTA.

Patient	Sex	Age	Age at onset	Number of stones	Stone composition	GFR ml/min	Concentration capacity mosm/kg	Phosphate/s mmol/l	Ca/s mmol/l	HCO_3 mmol/l	Ca/u mmol/24 h.
M.A.	F	23	17	2	-	82	820	1.1	2.4	25.5	3.5
S.B.	M	62	47	2	-	75	1 240	-	2.5	25	4
A.A.	F	35	23	8	Ca oxalate	-	860	0.8	2.4	22.5	5.0
I.H.	M	33	28	2	-	-	845	1.2	2.4	24.9	0.5
B.I.	M	56	51	3	-	73	880	0.7	2.5	25	5
E.J.	M	68	65	2	-	64	880	0.8	2.4	24	3
G.L.	M	52	50	2	-	75	940	1.1	2.4	24	5
H.L.	M	52	30	5	-	84	795	1.0	2.5	24	10
KE.L.	M	41	40	5	-	-	860	0.9	2.4	23.5	5
H.W.	M	40	28	4	-	135	980	1.0	2.4	22.5	7
P.A.	M	29	27	2	Ca oxalate + hydroxyapatite	66	730	1.0	2.5	26.5	1.5
I.A.	F	44	24	6	Ca oxalate + hydroxyapatite	89	840	0.8	2.3	27	7.5
C.B.	M	48	31	7	-	93	920	1.0	2.4	22	9.5
R.D.	M	50	47	8	-	71	1 050	1.0	2.4	22	5
S.E.	M	30	22	6	Ca oxalate + apatite	123	756	0.9	2.6	23	8
O.S.	M	37	18	10	Ca oxalate + apatite	78	800	0.8	2.5	25.5	10
T.M.	M	52	22	10	-	69	880	1.1	2.3	23.5	4
F.M.	M	33	16	8	-	79	880	0.9	2.2	21.5	8.5
B.S.	M	44	29	8	-	27	860	0.8	2.5	25	6
Å.P.	M	41	31	7	Ca oxalate	-	-	1.0	2.5	23	11
Y.S.	M	30	26	17	Ca oxalate	111	880	1.1	2.5	21.5	8
G.N.	M	46	32	8	Ca oxalate + phosphate	95	850	1.0	2.4	25	8
H.A.	M	49	30	20	-	119	800	0.9	2.5	26.5	7.5
T.B.	M	36	19	15	Ca oxalate	132	810	0.8	2.5	25	2
S.D.	M	36	25	11	Ca oxalate + apatite	106	1 050	1.1	2.4	26	5
R.K.	M	56	27	15	Ca oxalate + hydroxyapatite	82	758	1.0	2.4	25	5.5
R.W.	M	58	21	20	Ca oxalate	82	880	0.9	2.5	25	6.5
P.P.	M	39	31	20	Ca oxalate + phosphate	90	750	0.9	2.4	29	5
Å.O.	M	40	19	23	Ca oxalate	79	890	1.0	2.4	25	7
F.A.	M	36	25	3	Ca oxalate	-	925	1.0	2.4	24	11
B.O.	M	56	38	9	Ca oxalate + Ca phosphate	-	700	0.9	2.4	23	12
P.P.	M	32	29	4	Ca phosphate	-	830	1.1	2.5	24.5	6
SE.M.	M	43	31	7	-	-	1 060	0.7	2.4	25.5	4.5
A.B.	M	45	30	15	Ca oxalate + hydroxyapatite	-	740	0.9	2.4	23	10
PH.S.	M	34	27	5	Ca oxalate + hydroxyapatite	-	782	1.0	2.5	25.5	11

DISCUSSION

A large number of patients in this study (16/51) were found to have an impaired renal acidification capacity of varying degrees of severity. Such a high incidence of acidification defects in renal stone formers has not been reported in other studies (Williams & Chisholm, 1976; Barcelo & Pinto, 1973; Cochran, Peacock, Smith & Nordin, 1968). However, in their studies only patients with the complete and the incomplete form of RTA were diagnosed, in whom the urinary pH did not fall below 5.3. In our study we were able to diagnose also mild defects in cases in which the urinary pH was below 5.0 but there were pathological excretions of TA, NH_4^+ and phosphate. Proximal, tubular, acidification defects have previously not been reported in stone formers. For this diagnosis it is important to follow the degree of acidosis developed and to relate it to the urinary acidification.

Among our patients were some who had suffered from prolonged and severe stone disease with frequent recurrences and all of them had impaired acidification. They were investigated before this study but the cause of their stone formation was not found. This may have given our material a bias to the more severe forms of stone disease and may partly explain the high incidence of defects in our material.

This study showed a high incidence of acidification defects, 70 % among the women, as compared with 22 % among the men. Most of the women were young (20-40 years) and had an early onset of the stone disease. The adult form of renal tubular acidosis is said to be more common in women (about 70 %) (Seldin & Wilson, 1966). In many cases, a familial incidence of RTA was reported (Seldin & Wilson; Buckalew et al, 1974). The defect may vary in degree and in some cases appear only as nephrocalcinosis and nephrolithiasis. The disease is then genetically transmitted as an autosomal dominant trait. In at least two of our patients, the defect may have been primary and genetic, as there was a high incidence of cases of stones in their families.

Patients with a proximal acidification defect have an alkaline urine under normal conditions, because they lose bicarbonate ions in the urine. In cases of metabolic acidosis, when the filtered load of bicarbonate ions is reduced, the tubules are able to reabsorb all the filtered bicarbonate ions. At a higher degree of acidosis, there is a fall in the urinary pH below 5.0 and the excretions of TA, NH_4^+ and phosphate are also normal. In some other reports of

acid loading only urinary acidity was measured after the ingestion of ammonium chloride (Wrong & Davies; Williams & Chisholm). Cases in which the urinary pH is below 5.0 will all be assessed as having a normal acidification capacity and cases with bicarbonate wastage will not be diagnosed.

Proximal, tubular, acidification defect may be suspected to be caused by hyperparathyroidism (Muldowney, Caroll, Donohoe & Freaney, 1971; Siddiqui & Wilson, 1972). However, none of our patients showed any evidence of this disease as the serum calcium was normal.

Our method of relating the results from patients to a frame of reference, described in a previous study (Backman et al), seems necessary in order to detect both mild defects and proximal acidification defects. The latter should, however, be verified by bicarbonate loading.

It is of great importance to establish whether there are acidification defects in renal stone formers, as they are amenable to treatment (Tannen, 1975; Morris, Sebastian & MacSherry, 1972; Györy & Edwards; Young & Martin, 1972). Untreated patients with the incomplete form have a very high rate of recurrence of stones (Williams & Chisholm) and it has also been shown that the incomplete form may progress to the more severe, complete form with nephrocalcinosis (Buckalew et al, 1968). As regards our patients with mild defects, some of them have shown low levels of plasma bicarbonate, which suggests that these patients must be carefully followed up. Some of our patients with mild defects also showed hypercalciuria. Perhaps the criteria for defining the incomplete syndrome of tubular acidosis should be broadened, as some investigators (Buckalew et al) have suggested to include also milder forms of acidification defects.

The importance of the low excretion of phosphate seen in two of our patients is difficult to estimate, as their excretion of TA was normal. However, their normal excretion of TA could probably be explained by an increased excretion of hippuric acid (unpublished results).

Patients with renal tubular acidosis often have electrolyte disturbances, with increased potassium loss via the kidneys and hypokalaemia as a consequence. The concentration capacity is often reduced and glomerular filtration may be decreased. Table I shows that all patients with acidification defects had normal concentration capacities and only the patient with complete tubul-

ar acidosis (B.H.) had hypokalaemia.

The risk that any of the patients with defective acidification may have had chronic pyelonephritis with secondary acidification defects, as described by Cochran et al, is small, as clinical evidence was lacking, repeated urine cultures were negative and analysis of stones revealed calcium oxalate and/or calcium phosphate (Table I).

An important aspect of the development of acidification defects is the role of hypercalciuria. Some investigators have suggested that renal stone formers with hypercalciuria may develop an acidification defect. Boyce & Bradshaw (1961) noted incipient acidosis in patients with hypercalciuria and Nordin & Smith (1963) reported low excretions of citrate in urine, both findings suggestive of latent RTA. Recently, Buckalew et al suggested that hypercalciuria may be the primary event in hereditary, renal tubular acidosis. In two of our patients with incomplete and mild defects in acidification capacity, there was heavy hypercalciuria (15 mmol/24 hours), which suggests that other factors than metabolic acidosis are involved in the development of hypercalciuria. No patient without RTA showed this degree of hypercalciuria. Thus, account must be taken of the possibility of RTA developing in patients with heavy hypercalciuria and also in patients who are found to have low plasma bicarbonate levels, which may indicate an intermittent acidosis. If the suggestions of Buckalew et al are correct it seems even more important to treat the hypercalciuria in order to prevent the development of RTA.

In searching for the aetiology of acidification defects, many studies have been devoted to the enzyme carbonic anhydrase. Both in distal and in proximal tubular acidosis, confirmatory evidence has not been produced about the role of this enzyme. Mainly indirect methods, using acetazolamide, have been employed (Webster, Huth & Elkinton, 1960; Yaffe, Craig & Fellers, 1960; Soriano, Boichis, Stark & Edelman, 1967; Donckerwolcke, van Stekelenburg & Tiddens, 1970) and few investigators have studied the enzyme directly (Yaffe et al; Shapira, Ben-Youseph, Eyal & Russel, 1974). The methods used were probably too insensitive. The possibility of a defect of carbonic anhydrase does not appear unlikely, as this enzyme exists in an active form in both the proximal and in the distal tubules (Lönnerholm, 1972). A method of measuring the enzyme activity in urine and blood would be a great advance, especially if it were possible to detect latent cases of RTA among stone formers.

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BICARBONATE RE-ABSORPTION IN RENAL STONE PATIENTS WITH ACIDIFICATION DEFECTS

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ABSTRACT

A pathological renal response to ammonium chloride loading was found to be common in renal stone formers. In order to elicit further information about the impaired acidification capacity eight patients were investigated by bicarbonate loading. One patient with a mild acidification defect had normal tubular re-absorption of bicarbonate. Five of these eight patients with suspected proximal acidification defect and two patients with impaired distal acidification were found to have a low bicarbonate threshold and an increased, fractionated, bicarbonate excretion at a normal plasma bicarbonate level. Four of the patients with suspected, proximal, acidification defect reached a maximal re-absorption of bicarbonate (T_m) that was low, while three patients did not reach a T_m. These three factors indicate increased bicarbonate wastage from the kidneys. A constantly alkaline urine, such as exists in patients with proximal acidification defect may give rise to an increased risk of the precipitation of renal stones - primarily calcium-phosphate stones.

Key words: Renal tubular acidosis; renal stone disease; bicarbonate excretion

INTRODUCTION

Two main types of renal tubular acidosis exist, in which the defects are considered to be primarily localized to either the proximal or the distal renal tubules. In the "classical", distal, tubular acidosis (RTA, type I), the clinical picture is characterized by metabolic acidosis, increased urinary pH, bicarbonaturia and a reduced excretion of titratable acid (TA) and ammonium ions (Wrong & Davies, 1959; Seldin & Wilson, 1966; Morris, Sebastian & MacSherry, 1972). The bicarbonaturia that exists is usually small at normal plasma bicarbonate levels (Morris et al). A relatively small amount of alkali (1-3 mmol/kg/day) is required in the majority of cases to correct the acidosis (Seldin & Wilson; Morris et al). Some patients have, however, been shown to be "alkali-resistent" as their acidosis could not be corrected even by large doses of alkali (MacSherry, Sebastian & Morris, 1972). These patients had a bicarbonate excretion of between 5 and 10 per cent of the filtered amount at normal plasma bicarbonate levels. The classical RTA with bicarbonate loss can, however, be distinguished physiologically from the proximal tubular acidosis (RTA, type II) (MacSherry et al). In the latter condition, there is a considerable bicarbonaturia, which at normal plasma bicarbonate levels exceeds 15 per cent of the filtered quantity (Morris et al). The urinary pH is therefore high and decreases below pH 5.0 only when the patient develops a high degree of acidosis (Morris et al; Donckerwolcke, van Stekelenburg & Tiddens, 1970; York & Yendt, 1966). The excretion of TA and NH_4^+ are also normal, which indicates normal, distal, tubular function.

We have previously reported studies of acidification capacity in idiopathic renal stone formers (Backman, Danielson & Sohtell, 1976). Among 51 patients with recurring, calcium-containing stones, 31 per cent had some acidification defect, including six patients with suspected, proximal, tubular acidosis. None of the patients had hyperparathyroidism as a cause of their acidification defect. Five of the six patients had a severe stone disease with a history of frequent recurrences and many operations. Two of them were also found to have a dilution defect, described in a previous report (Backman, 1976). This proximal acidification defect has previously been considered uncommon (Morris et al) and has not been described in patients with renal stones. The aim of this study was to further study both the proximal and the distal acidification defects by the method of bicarbonate loading.

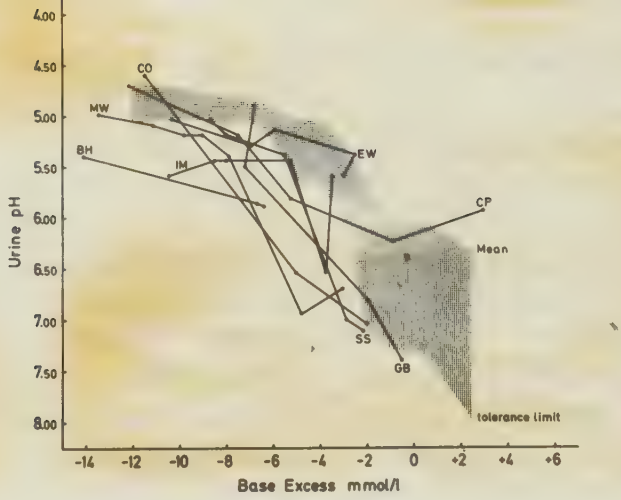

Fig. 1. Urinary pH in relation to the BE in the ammonium chloride loading of eight patients with acidification defects

MATERIAL

Twelve patients with renal stones (seven women and five men) whose renal acidification capacities had previously been investigated by ammonium chloride loading, were examined (Backman et al). Five patients had suspected, proximal, tubular acidosis. Three patients had distal acidification defects, of whom one woman (I.M.) had incomplete RTA and another woman (E.W.) was found to have milder defects in the excretion of titratable acid and ammonium ions. The third patient (B.H.) had the complete form of RTA and her previously noted hypokalaemia was corrected before this examination. Fig. 1 shows the urinary pH in relation to the BE in the ammonium chloride loading test on the eight patients with acidification defects. As a control, four patients with normal acidification capacities were examined. A short summary of some clinical and laboratory data concerning the patients with acidification defects will be found in Table I.

METHODS

The examination was commenced in the morning. The patients had received ammonium chloride tablets in a dose of approximately 100 mmol/m^2 body surface the previous evening. They were allowed to eat as usual and approxim-

Table I. Some clinical and laboratory data from seven patients with defects of bicarbonate re-absorption

Patient	Sex	Age	Duration years	Stone composition	Number of stones	Concentration capacity mOsmol/kg	Ca/u mmol/ 24 hours
Distal acidification defects							
E.W.	F	22	2	Hydroxyapatite	6	900	15.0
I.M.	F	22	2	Hydroxyapatite	8 - 10	940	3.0
B.H.	F	29	5	Ca oxalate	25	800	5.0
Proximal acidification defect							
C.P.	F	27	17	Ca oxalate + hydroxyapatite	8 - 9	1 000	6.5
M.W.	F	34	4	Ca oxalate + hydroxyapatite	7 - 8	1 090	3.0
G.B.	F	35	2	Hydroxyapatite	3	690	8.0
S.S.	M	47	26	Ca oxalate	Several 100	810	7.0
C.O.	M	49	40	-	100	1 242	4.5

ately 100 ml of fluid per hour were given during the investigation. The glomerular filtration was measured with inulin, which was given as a priming dose i.v., followed by continuous infusion of a quantity calculated to yield a plasma concentration of approximately 15 mg/100 ml (Pitts, 1970). After two or three inulin clearance periods of 30 minutes each, a continuous infusion of 30 mmol/hour bicarbonate was given. Bicarbonate was also given as priming doses calculated in the following manner: 0.3 x kg b.w. x desired increase of the base excess (Nahas, 1969), whereafter the plasma bicarbonate level was calculated as being low, normal or high.

After an equilibration period of one hour after the priming doses of bicarbonate, the clearance was measured during two 30 minute periods. The investigation was usually terminated at a plasma bicarbonate level of 30 mmol/l or higher, at which level it could be assumed that the patients had reached a T_m. The amount of fluid administered was approximately 280 ml/hour but, towards the end of the examination, was sometimes slightly in excess of this, as extra bicarbonate was administered to yield a more rapid alkalosis. Urine was collected via an indwelling urinary catheter. Mild suprapubic pressure was used to ensure that the bladder was emptied as completely as possible.

Arterialized capillary samples were taken before the bicarbonate loading and in the middle of each clearance period and stored in iced water. The acid base status was analysed within five minutes. Blood samples for measurement of plasma inulin were taken from an indwelling, peripheral, venous catheter. Urine samples for measurement of pH and P_{CO_2} were taken by syringe from the urinary catheter. The needle was immediately thereafter sealed with a rubber bung and the syringe stored in ice. The urinary pH was analysed within five minutes and P_{CO_2} and HCO_3^- within half an hour to one and a half hours. The urine was also analysed for inulin, sodium and potassium.

ANALYTICAL METHODS

Inulin was measured spectrophotometrically with diphenylamine (Hogeman, 1948). The urinary pH was measured with a glass electrode G 297 and a calomel electrode K 497 at 38° C (Radiometer, Copenhagen). The P_{CO_2} in the urine was measured by the Astrup equilibration method at CO_2 concentra-

tions of 3 and 11 per cent (Radiometer, Copenhagen). Sodium and potassium were measured in a flame photometer. The capillary samples were analysed for acid base status in an IL Automatic pH/blood-gas analyser Model 313 (Instrumentation Lab., Mass) or by the Astrup equilibration method (Radiometer, Copenhagen).

CALCULATIONS

The urinary HCO_3^- was calculated from the urinary pH and P_{CO_2}, according to the Henderson-Hasselbalch equation:

$$\text{Urinary pH} = pK + \log HCO_3^- - \log s\, P_{CO_2}$$

where pK is assumed to be $6.33 - 0.5\sqrt{[Na^+] + [K^+]}$ (Slatopolsky, Hoffsten, Purkerson & Bricker, 1970). s is the solubility coefficient for CO_2 in urine and is assumed to be 0.03 (Slatopolsky et al).

RESULTS

Normal, renal re-absorption of bicarbonate

Fig. 1 shows the results from the four subjects with normal acidification capacities. These four individuals began to excrete bicarbonate in their urine at a plasma bicarbonate level of 24-26 mmol/l. Two of them reached a T_m of 2.6 mmol HCO_3^- per 100 ml GFR (Fig. 2). This is within the normal range according to the figures for the normal range (2.6 - 2.8 mmol HCO_3^- per 100 ml GFR) given by Morris et al. Two patients did not reach a T_m, but re-absorption was 2.6 and 2.8 mmol HCO_3^- per 100 ml GFR at plasma bicarbonate levels of 32.8 and 29 mmol/l respectively (Fig. 2). The fractionated excretion of filtered bicarbonate ($C_{HCO_3^-}/C_{In}$) at a normal plasma bicarbonate level was 2-4 per cent for all four subjects (Table II), which is within the normal range (Morris et al). One patient with a mild, distal, acidification defect (E.W.) showed the same pattern and had a bicarbonate re-absorption of 2.8 mmol HCO_3^- per 100 ml GFR at a plasma bicarbonate level of 29 mmol/l. These five patients were judged to have a normal re-absorption of bicarbonate in the kidney.

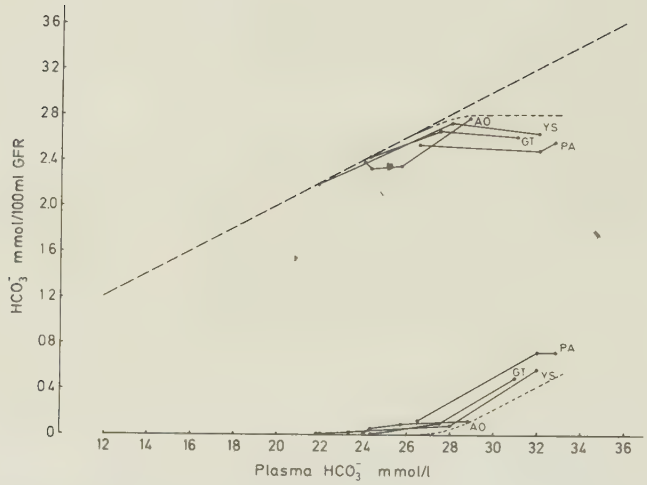

Fig. 2. The relationship between plasma concentration, renal tubular re-absorption and urine excretion of bicarbonate in four patients with normal acidification capacities

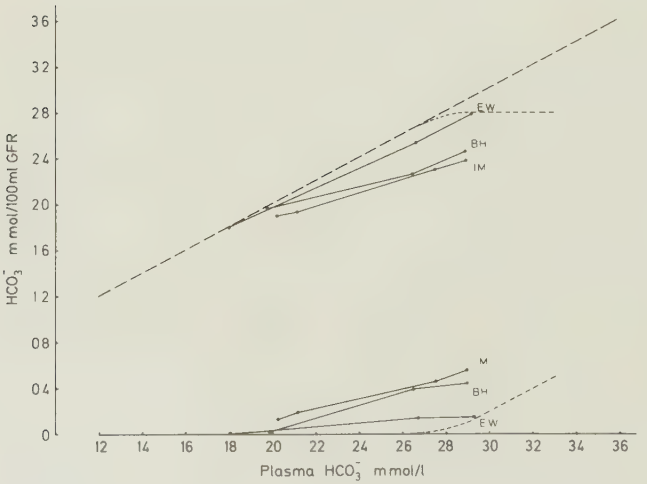

Fig. 3. The relationship between plasma concentration, renal tubular re-absorption and urine excretion of bicarbonate in three patients with distal acidification defects

Table II. Levels of bicarbonate in plasma and urine and GFR and V at normal plasma bicarbonate levels and alkalosis

	Before bicarbonate loading	Normal acid-base status					Alkalosis					Acidification capacity
Patient	HCO_3^-/s mmol/l	HCO_3^-/s mmol/l	$C_{HCO_3^-}/C_{In}$ %	V ml/min	V/GFR %	GFR ml/min	HCO_3^-/s mmol/l	$C_{HCO_3^-}/C_{In}$ %	V ml/min	V/GFR %	GFR ml/min	
G.T.	22.8	24.3	0.1	1.5	2	92	27.5	3	2.9	2.7	88	normal
Å.O.	19.5	24.3	2.0	1.7	2	103	28.8	5	3.3	2.6	112	normal
Y.S.	18.2	23.3	0.01	1.0	1	98	28.0	4	2.4	2.4	89	normal
P.Å.	18.5	26.5	4	1.5	2	77	32.0	21	3.5	4.0	79	normal
E.W.	14.7	26.7	4	3.3	2.5	133	29.3	5	2.9	2.0	138	Type 1
B.H.	11.6	19.8	0.1	0.6	1	70	26.5	18	1.6	2.0	77	Type 1
I.M.	12.9	21.2	7	1.1	1	101	27.5	17	4.2	3.0	126	Type 1
C.O.	7.0	19.0	21	2.4	2	107	27.5	24	3.9	4.0	95	Type 2
G.B.	17.5	21.8	9	2.1	2	91	28.0	32	4.7	4.5	101	Type 2
S.S.	13.5	22.3	13	1.7	2	76	29.5	35	5.8	6.6	87	Type 2
C.P.	13.4	22.5	3	1.7	2	103	25.0	16	2.7	3.0	96	Type 2
M.W.	10.6	20.3	1	2.0	2	116	25.5	18	2.4	2.0	106	Type 2

Distal, tubular, acidification defect

Two patients (B.H. and I.M.) with distal acidification defects began to excrete small quantities of bicarbonate in the urine at a plasma bicarbonate level of 20 mmol/l. Both patients continued to re-absorb bicarbonate at a plasma bicarbonate level of 29 mmol/l and did not reach a T_m (Fig. 3). The fractionated excretion of bicarbonate in the urine at normal and subnormal plasma bicarbonate levels was, however, greater than normal (Table II).

Proximal, tubular, acidification defect

Four of the five patients with suspected, proximal, acidification defects reached a T_m between 1.8 and 2.1 mmol HCO_3^- per 100 ml GFR (Fig. 4). The fifth patient did not reach a T_m but the investigation in this case was terminated at a plasma bicarbonate level of 25.5 mmol/l. All the patients in this group began to excrete bicarbonate in the urine at a plasma bicarbonate level between 18 and 22 mmol/l. The fractionated bicarbonate excretion at normal and subnormal bicarbonate values in the plasma was raised in all the patients, especially so in three patients (Table II). These findings agree with those in studies of proximal tubular acidosis (Morris et al).

An increase in GFR with increased bicarbonate administration was seen in only one patient (I.M.) (Table II). A considerably increased bicarbonate excretion in the urine was noted in four patients during alkalosis and in all these patients an increased diuresis was noted (Table II).

Fig. 5 shows that bicarbonate excretion in urine began when the pH in the urine was about 6.4 and increased thereafter rapidly with increased pH.

DISCUSSION

We have previously reported that a large number of idiopathic renal stone formers had a pathological response to ammonium chloride loading (Backman et al). Some patients appeared to have defects in the distal tubular acidification, while others were found to have a delayed renal response to the ammonium chloride induced acidosis with otherwise normal acidification. These patients were suspected of having defects in the proximal tubules with a lowered renal bicarbonate threshold. This study has provided con-

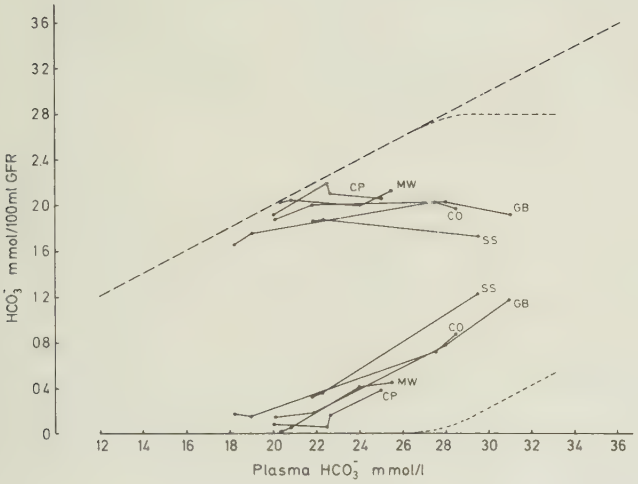

Fig. 4. The relationship between plasma concentration, renal tubular re-absorption and urine excretion of bicarbonate in five patients with suspected proximal acidification defects

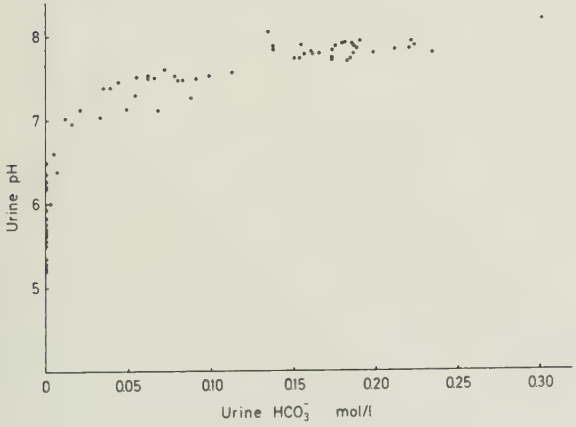

Fig. 5. The relationship between the pH in the urine and the excretion of bicarbonate

firmatory evidence in favour of this hypothesis. Also patients with an impaired distal acidification were found to have incomplete re-absorption of bicarbonate. All the patients with a normal response to ammonium chloride loading were found to have a complete re-absorption of bicarbonate until the plasma bicarbonate levels exceeded 24-26 mmol/l.

The diagnosis of proximal acidification defect was made because of the low bicarbonate threshold, the decreased T_m and the increased, fractionated, bicarbonate excretion ($C_{HCO_3^-}/C_{In}$) at normal plasma bicarbonate levels (Morris et al). Other factors can, however, influence the re-absorption of bicarbonate and give falsely increased or decreased values. Expansion of the extracellular volume and water diuresis cause a restriction of the sodium re-absorption in the proximal tubules, with a coincident reduction in the re-absorption of bicarbonate (Slatopolsky et al; Morris et al). No patient in this study had polyuria and all the patients had normal concentration capacities (Backman) (Table I). The urine volume towards the end of the investigation increased somewhat, most markedly, however, in four patients with bicarbonate loss (Table II). The urine volume did not exceed 4-6 per cent of the GFR, at which the fractionated bicarbonate excretion is only insignificantly greater than at a V/GFR of 1-2 per cent (Morris et al).

The five patients with proximal acidification defect had under normal conditions normal or only mildly reduced bicarbonate levels in plasma (Table I). To enable these plasma bicarbonate levels to remain constant, bicarbonate mobilization from bone has been proposed, with an increased risk of osteomalacia (York & Yendt).

Proximal tubular acidosis caused by defective bicarbonate re-absorption in the renal tubules was first described by Lamy, Frézal, Rey & Larsen (1962) in a child suffering from Fanconi's syndrome and has lately been described primarily as a component of a more complex tubular dysfunction (Soriano, Boichis, Stark & Edelmann, 1967; Morris et al). Sporadic cases, primarily in children, have been reported with no signs of other diseases (Soriano et al; Donckerwolcke et al). In adults hyperparathyroidism is probably the commonest cause of proximal acidification defect, which is reversible in these cases (Muldowney, Carroll, Donohoe & Freany, 1971). In our patients, however, there was no evidence of hyperparathyroidism as a cause of their acidification defect as all of them were found to have normal serum-calcium levels.

Despite several similarities in the clinical picture in the two types of acidification defects (metabolic acidosis, hypercalciuria, risk of osteomalacia), it is not known why renal stone formation is not seen more commonly in both the primary and the more complex proximal tubular dysfunctions. Sporadic reports of renal stones and proximal tubular defects have been published, in cases of, Balkan nephropathy, which is considered to be a tubular disease affecting primarily the proximal tubules (Hall, Gaon, Griggs, Piscator, Popović, Vasilović & Zimonjic, 1967; Kazantzis, 1967). These patients also have hypercalciuria and one case even nephrocalcinosis (Kazantzis). Patients with cadmium nephropathy have also in a few cases developed renal stones (Adams, Harrison & Scott, 1969). An interesting comparison can be made with the iatrogenically induced, proximal, tubular acidosis and renal stone formation that has been noted in several patients who have received long-term treatment with acetazolamide (Mackenzie, 1960; Parfitt, 1969; Wallace, MacDiarmid & Reeder, 1974). Stone formation in these patients is considered to be related in part to the alkaline urine and in part to the significantly reduced excretion of citrate in the urine (Mackenzie). Citrate is considered to complex-bind calcium and an increased citrate excretion should thereby reduce the tendency to stone formation. The citrate excretion in the urine has not yet been examined in our patients.

It is at present impossible to judge whether the proximal acidification defect in these patients is primary or secondary. A constantly alkaline urine, however, should increase the risk of precipitation of calcium phosphate primarily. Several of our patients were also found to have phosphate in their stones (Table I).

The constantly alkaline urine can also be considered to increase the risk of urinary tract infections and thereby further increase the tendency to stone formation. Under these conditions, the renal injury may progress so that even the distal tubules become involved. A high frequency of distal acidification defects has been found in cases of chronic urinary tract infections with renal stones (Cochran, Peacock, Smith & Nordin, 1968; Sommerkamp & Schwerk, 1973). Our patients, however, showed no signs of chronic pyelonephritis.

The results of this study also showed that patients with impaired distal acidification can have bicarbonate wastage. It has been suggested that in some patients with classic RTA an impaired re-absorption of bicarbonate in the

proximal tubules is possible (Morris et al).

Confirmatory evidence of affections of the proximal tubules in renal stone formers was given in this study. The patients will be followed up and investigated for further possible affections of the tubules.

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URINARY EXCRETION OF β_2-MICROGLOBULIN IN RENAL STONE PATIENTS UNDER NORMAL CONDITIONS AND DURING ACIDOSIS AND ALKALOSIS

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ABSTRACT

The urinary excretion of β_2-microglobulin was studied under normal conditions and during acidosis and alkalosis in 65 patients with renal stones. Eleven patients were studied under two of these conditions. Four out of 51 patients examined under normal conditions had an increased excretion of β_2-microglobulin in their urine. Four out of five patients with distal acidification defects were found to have an increased excretion of β_2-microglobulin during induced acidosis. During alkalosis, four out of eight patients with acidification defects increased their excretion of the protein. The tubular proteinuria that could be provoked during acute acidosis and alkalosis was considered to be secondary to changes in the acid-base status and may indicate a renal tubular defect.

Key words: Tubular proteinuria; β_2-microglobulin; renal stone disease

INTRODUCTION

The excretion of proteins with low molecular weights (such as insulin, lysozyme and β_2-microglobulin) is increased in the urine in cases of tubular proteinuria (Maack, 1975). β_2-microglobulin has a molecular weight of 11 800 (Berggård & Bern, 1968). It is filtered through the glomeruli and reabsorbed almost totally in the tubules under normal conditions (Evrin & Wibell, 1972). An increased excretion in the urine occurs in cases of uraemia and also in tubular diseases in which glomerular filtration is maintained (Berggård & Bearn; Johansson & Ravnskov, 1973). The demonstration of tubular proteinuria has been found to be a sensitive test in the diagnosis of early stages of tubular disease, such as Balkan nephropathy (Hall, Gaon, Griggs, Piscator, Popović, Vasilović & Zimonjic, 1967) and juvenile nephronophthisis (Lindstedt & Lindstedt, 1973), as well as in demonstrating the nephrotoxic effect in cases of cadmium poisoning (Piscator, 1966).

β_2-microglobulin has been shown to be a sensitive method of demonstrating tubular proteinuria also in mild affections of the tubules (Kjellberg, Piscator & Castenfors, 1971). The presence of tubular defects in patients with renal stones has rarely been investigated. An increased excretion of insulin in 14 of 18 patients examined has, however, been demonstrated (Ching, Karam, Choy, Kolb, Grodsky & Forsham, 1972) and lysozyme in the urine in four out of five patients investigated (Kregzde, Lambert & Davidson, 1969). The importance of these findings was, however, not clear and the presence of other tubular defects was not reported. As we had previously described renal defects in patients with renal stones, primarily in the form of acidification defects (Backman, Danielson & Sohtell, 1976 b), we considered it of interest to examine these patients for further tubular affections.

MATERIAL AND METHODS

1. Investigation under normal conditions

Fifty-one patients, 14 women and 37 men aged between 22 and 67 years with recurrent renal stones, were investigated. Table I shows some of the clinical and laboratory findings in these patients. Many of them were found to have tubular affections with impaired acidification and dilution capacities.

Table I. Some clinical and laboratory findings in 51 patients with recurring renal stones

	Hypercalciuria (>6 mmol/24 hours)	Ca/s (<2.6 mmol/l)	Concentration capacity (≥800 mOsmol/kg)	Dilution defect	RTA	Chron. pyelonephr.	Cystin-uria	Uric acid stone
F	3	14	13	4	7	1		1
M	21	37	34	3	6	1	1	
Total	24	51	47	7	13	2	1	1

Table II. Some clinical and laboratory findings in 15 patients with recurring stones, investigated by ammonium chloride loading

	n	Hypercalciuria (>6 mmol/24 hours)	Ca/s (< 2.6 mmol/l)	RTA	Chron. pyelonephr.
F	4		4	1	
M	11	4	11	4	2
Total	15	4	15	5	2

The excretion of β_2-microglobulin in a 24-hour urine sample was examined. Because β_2-microglobulin is degraded at pHs below 5.5, the patients were given 5 g $NaHCO_3$ at intervals during the day and 5 g in the evening. The urine was analysed for pH and creatinine. Serum samples for the determination of creatinine and β_2-microglobulin were taken in the morning, directly after urine collection.

2. Investigation during ammonium chloride loading

Ten patients with idiopathic renal stones but with normal acidification capacities were investigated. Five patients with distal acidification defects, two of whom had chronic pyelonephritis (K.B. and R.A.) and one a medullary sponge kidney (J.S.) were also studied. Table II shows some of the clinical and laboratory findings in these 15 patients.

During the ammonium chloride loading test (dose of ammonium chloride: 150 $mmol/m^2$ body surface), which has been described previously (Backman, Danielson & Sohtell, 1976 a), urine was collected for the measurement of β_2-microglobulin during increasing degrees of acidosis. Two or three drops of 2 M NaOH were added to each sample to prevent the breakdown of β_2-microglobulin. NaOH was not added to the urine from the five patients with renal tubular acidosis, as the urine pH was greater than 5.5.

3. Investigation following bicarbonate loading

Twelve patients, with renal stones, seven of whom had signs of bicarbonate wastage in the urine and five had normal bicarbonate re-absorption, were investigated. All these patients have been described in an earlier report (Backman, Danielson & Sohtell, 1976 c). Some clinical and laboratory findings are

Table III. Some clinical and laboratory findings, in 12 patients with renal stones, subjected to bicarbonate loading

	n	Hypercalciuria (>6 mmol/24 hours)	Ca/s (< 2.6 mmol/l)	Concentration capacity (≥ 800 mOsmol/kg)	Dilution defect	RTA	Uric acid stone
F	7	2	7	6	3	6	1
M	5	3	5	4	0	2	
Total	12	5	12	10	3	8	1

shown in Table III.

Five healthy subjects with no history of renal disease were also investigated. They were given 10 g $NaHCO_3$ on the evening before the investigation and on the following morning a further 5 g $NaHCO_3$. Arterialized capillary samples for the analysis of acid-base status were taken and urine was collected during 2 hours for analysis of β_2-microglobulin.

The twelve patients were loaded with bicarbonate intravenously, after previous acidification with ammonium chloride (100 mmol/m^2 body surface). Bicarbonate was given in priming doses of about 30-90 mmol and in continuous infusions of 30 mmol/hour. Urine was collected during increased alkalosis for the analysis of β_2-microglobulin.

To prevent bacterial growth in the samples from all three investigations, 1 ml of 5 % sodium azide (NaN_3) was added to each litre urine. The samples were stored in a deep-freeze before analysis.

Analytical method

β_2-microglobulin was analysed by radio-immuno-assay technique (Phadebas, AB Pharmacia Diagnostics, Uppsala, Sweden). Normal values in serum were: 1.1 - 2.4 mg/l, and in urine 30 - 370 µg/24 hours. From these figures, the clearance of β_2-microglobulin was calculated to be about 0.02 - 0.10 ml/min.

RESULTS

1. Urinary excretion of β_2-microglobulin under normal conditions

Three patients (B.H., P.P. and F.M.) had increased urinary excretions of β_2-microglobulin and one patient (Y.S.) had values in the high normal range (Table IV). The clearance of β_2-microglobulin was also high and, in relation to the creatinine clearance, the values for these four patients were greater than has been reported as normal (Johansson & Ravnskov). Three of the four patients had hypercalciuria and the fourth patient had distal tubular acidosis. Table V shows the means, standard deviations and ranges for the other 47 patients with normal excretions. β_2-microglobulin in serum was found to

Table IV. Values of β_2-microglobulin in 11 patients with increased excretions under normal conditions and during acidosis and alkalosis

Patient	Sex	Age	Diagnosis	Ca/urine mmol/24 hours	β_2-microglobulin/s mg/l	β_2-microglobulin/urine µg/24 hours	β_2-microglobulin clearance ml/min	β_2-microglobulin clearance / creatinine clearance	β_2-microglobulin/u at max. acidosis µg/hour	β_2-microglobulin/u at max. alkalosis µg/min	HCO_3^-/s mmol/l
Y.S.	M	30	Hypercalciuria	7.0	2.5	352	0.10	0.15	-	0.112	21.5
B.H.	F	30	RTA, type 1	5.0	2.1	895	0.30	0.26	(65)*	1.255	17.5
P.P.	M	32	Hypercalciuria	6.3	1.6	1 215	0.53	0.47	-	-	24.5
F.M.	M	34	Hypercalciuria	10.0	1.7	407	0.17	0.14	-	-	21.0
S.S.	M	48	RTA, type 2	7.0	2.1	220	0.07	0.04	(22)*	2.761	23.0
C.O.	M	50	RTA, type 2	5.0	1.8	155	0.06	0.04	(155)*	0.994	22.0
I.M.	F	23	RTA, type 1	2.5	1.5	189	0.09	0.07	34	1.450	22.0
P.A.	M	30	Normal	1.0	-	-	-	-	-	0.728	26.5
J.S.	M	19	Med.sponge kidney	14.0	-	-	-	-	7 544	-	22.0
H.P.	M	52	RTA, type 1	15.0	1.4	145	0.07	0.05	25	-	20.5
R.A.	M	22	Chronic pyelonephr.	0.5	-	-	-	-	25	-	24.5

* Values obtained during acidosis preceding bicarbonate loading.

Table V. Means, standard deviations and ranges for B_2-microglobulin in the serum and urine of 47 patients with normal values of B_2-microglobulin

	B_2-microglobulin/s mg/l	B_2-microglobulin/u g/24 hours	Clearance of B_2-microglobulin ml/min	Clearance of B_2-microglobulin / creatinineclearance
Mean	2.06	114	0.05	0.04
SD	0.56	64	0.02	0.02
Range	0.7 - 2.3	30 - 317	0.01 - 0.11	0.01 - 0.11
n	47			

Table VI. Urinary excretion of β_2-microglobulin related to the base excess in five healthy subjects after ingestion of 15 g $NaHCO_3$

Subject	β_2-microglobulin in urine µg/hour	Base excess mmol/l
1	9.5	+2.2
2	5.1	+4.7
3	6.5	+5.4
4	1.8	+7.8
5	6.8	+2.8

be just above the normal range in three patients (3.4 and 2.5 mg/l).

2. Urinary excretion of β_2-microglobulin during acidosis

During acidification, ten patients with normal renal acidification capacities had normal excretions of the protein (normal value calculated from a 24-hour excretion not exceeding 16 µg per hour) (Fig. 1). In comparison with the ten patients, four with a renal acidification defect (RTA) had increased excretions that in two cases increased with increasing acidosis (Fig. 2). The patient with the greatest increase had a prolonged acidosis, as extra ammonium chloride was given after ten hours, in order to see whether the urinary pH would decrease with a higher degree of acidosis. The maximal level of β_2-microglobulin was found after 24 hours and is a mean of three different urine collections.

3. Urinary excretion of β_2-microglobulin during alkalosis

Five healthy subjects showed an excretion of 2-8 µg of β_2-microglobulin/hour during alkalosis (Table VI), which was within the normal range, calculated from the 24 hour excretion. Seven patients who were subjected to bicarbonate loading also had normal excretions, which, however, increased somewhat with increasing degrees of alkalosis (Fig. 3) (The normal level calculated from the 24-hour excretion should not exceed 260 ng/min). Three of these patients had signs of bicarbonate wastage in their urine (Backman et al 1976 c). One patient (P.A.), who had a normal renal re-absorption of bicarbonate, showed a considerably greater increase in the excretion of β_2-micro-

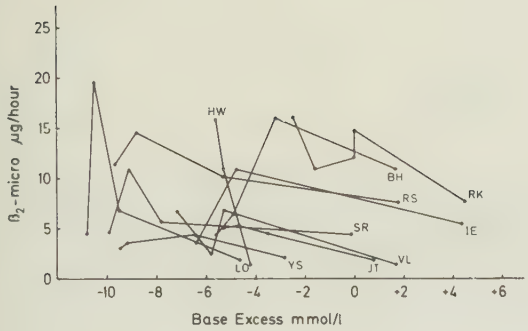

Fig. 1. Urinary excretion of β_2-microglobulin during acidosis in ten patients with normal acidification capacities

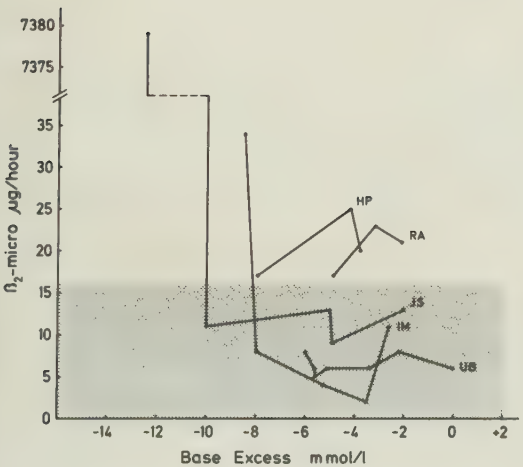

Fig. 2. Urinary excretion of β_2-microglobulin during acidosis in five patients with distal acidification defects

globulin with increasing alkalosis. Four patients, all of whom had acidification defects (RTA), two of distal type (one case had the complete and the other the incomplete form) and two of proximal type, had increased excretions during acidosis and increased their excretion with increasing bicarbonate administration and alkalosis (Fig. 4).

DISCUSSION

In previous studies of tubular proteinuria, it has been shown that there is a close correlation between the glomerular filtration rate and the clearance of β_2-microglobulin (Johansson & Ravnskov). An increased urinary excretion of the protein may only reflect a renal insufficiency when the glomerular filtration rate is decreased. On the other hand an increased urinary excretion of the protein may also reflect selective tubular damage with an impaired re-absorption of the protein in the presence of a normal filtration rate. Thus, it seems very important to report not only the urinary excretion but also the relation between the proteinclearance and the filtration rate. In our patients, the creatinine clearance was normal and the increased excretion of β_2-microglobulin in four patients thus seems to have been due to a disruption of the glomerulotubular balance for the protein and to indicate isolated damage to the tubules. Another advantage of relating the two clearances to each other is that minor inaccuracies in the urine-sampling will not influence the quotient, as volume cancels out volume in the formula.

We have previously described tubular affections in renal stone formers mainly as impairment of the acidification and dilution capacities (Backman et al, 1976 b, c; Backman, 1976). These defects were considered to be of pathogenetic significance for the stone formation. Further evidence of tubular defects in some of the renal stone formers was demonstrated in this study. Eleven out of 65 patients were found to have increased urinary excretions of β_2-microglobulin in normal conditions or during acidosis and alkalosis. Eleven patients were studied under two conditions and only one under all three conditions. Five of these patients did not have any other signs of tubular dysfunction or hypokaliemia, but three of them with tubular proteinuria under normal conditions had hypercalciuria. The demonstration of tubular proteinuria in renal stone formers appears to be important as it indicates that tubular affections may exist with no signs of tubular dysfunction. In other tubular diseases, it was shown that tubular proteinuria developed

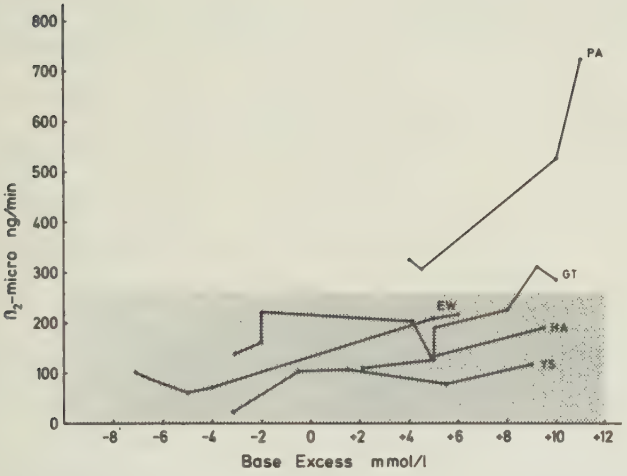

Fig. 3. Urinary excretion of β_2-microglobulin during alkalosis in five patients with normal acidification capacities

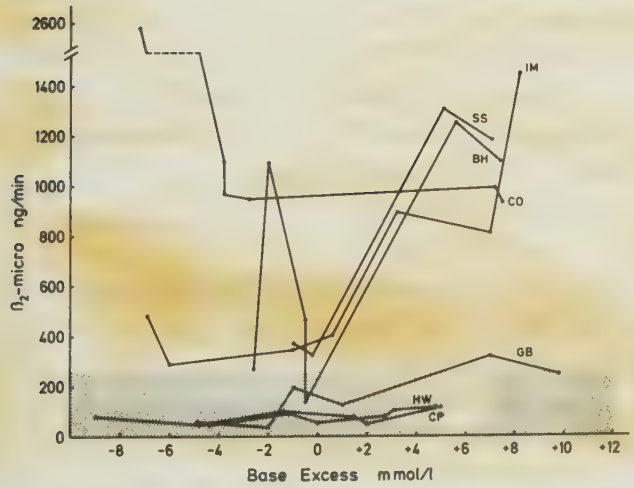

Fig. 4. Urinary excretion of β_2-microglobulin during alkalosis in seven patients with acidification defects

before the impairment of tubular function (Lindstedt & Lindstedt; Hall et al). Thus the possibility of a progress of the tubular affection in our patients must be considered. As three of our patients also had hypercalciuria a development of an acidification defect may be possible. Renal stone formers with hypercalciuria have previously been shown to develop renal tubular acidosis (Buckalew, Purvis, Shulman, Herndon & Rudman, 1974).

In four of the other patients, the tubular proteinuria could be demonstrated only when acute acidosis or alkalosis was induced. All four patients had acidification defects and it is possible that their tubules are especially sensitive to changes in acid-base status. Earlier investigations of β_2-microglobulin excretion in acidosis indicated that the tubular proteinuria was probably due to hypokaliemia (Kjellberg et al). In their study, it was shown that the tubular proteinuria disappeared after treatment with potassium but not after treatment with bicarbonate. Chronic hypokaliemia is known to produce tubular defects with histopathological changes localized in the proximal tubules (Hamburger, 1968). There were, however, no signs of hypokaliemia in our patients.

The tubular proteinuria that was found during acidosis and alkalosis may be considered to be functional and reversible, as none of the patients, except one, had tubular proteinuria under normal conditions. The tubular functions of our patients appeared to be especially vulnerable to changes in acid-base status. This may also be considered a sign of a tubular defect that could be demonstrated only during acidosis and alkalosis. As several of the patients had sometimes been shown to have somewhat low plasma bicarbonate levels (Table IV), it is possible that an intermittent acidosis may occur which would possibly increase the risk of irreversible tubular damage (Buckalew, MacCurdy, Ludwig, Chaykin & Elkinton, 1968).

Though the results are preliminary, they illustrate the fact that several renal tubular defects may be found in patients with recurring renal stones. This indicates that the kidneys may be affected in cases of recurrent kidney stone disease also in the absence of tubular dysfunction. The incidence of tubular defects in these patients is still not clear and must be studied in a larger patient material. Studies should also be carried out to see whether the tubular proteinuria is of any importance in the process leading to the stone formation.

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Financial support from the Swedish Medical Research Council (14X-2329, K 75-29X-4629) and Harald Jeanssons Stiftelse.

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